(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 February 2003 (20.02.2003)

PCT

(10) International Publication Number
WO 03/014377 A2

(51) International Patent Classification[7]: C12Q 1/00

(21) International Application Number: PCT/CA02/01214

(22) International Filing Date: 5 August 2002 (05.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/310,272 7 August 2001 (07.08.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

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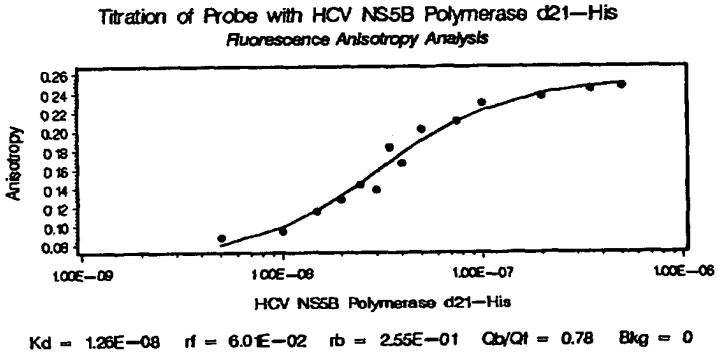
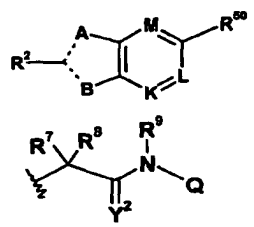
(54) Title: DIRECT BINDING ASSAY FOR IDENTIFYING INHIBITORS OF HCV POLYMERASE





(I)

(57) Abstract: A method for identifying compounds binding to HCV polymerase comprising the steps of:contacting said HCV polymerase or an analog thereof with a probe formula I:wherein A is O, S, N, NR1, or CR1, wherein R1 is defined herein;----- represents either a single or a double bond;R2 is selected from: H, halogen, R21, OR21, SR21, COOR21, SO2N(R22)2, N(R22)2, CON(R22)2, NR22C(O)R22 or NR22C(O)NR22 wherein R21 and each R22 is defined herein;B is NR3 or CR3, wherein R3 is defined herein;with the proviso that, when A is not N, then one of A or B is either CR1 or CR3, K is N or CR4, wherein R4 is defined herein;L is N or CR5, wherein R5 has the same definition as R4 defined above;M is N or CR7, wherein R7 has the same definition as R4 defined above;R5 is C(Y1)Z wherein Y1 is O or S; and Z is N(R6a)R6 or OR6, wherein R6a is H or alkyl or NR61R62 wherein R61 and R62 are defined herein; and R6 is H, alkyl, cycloalkyl, alkenyl, Het, alkyl-aryl, alkyl-Het;or R6 is wherein R7 and R8 and Q are as defined herein; Y2 is O or S;R9 is H, (C1-6 alkyl), (C3-7)cycloalkyl or (C1-6)alkyl-(C3-7)cycloalkyl, aryl, Het, (C1-6)alkyl-aryl or (C1-6)alkyl-Het, all of which optionally substituted with R90; or R9 is covalently bonded to either of R7 or R8 to form a 5- or 6-membered heterocycle;or a salt thereof; where the probe comprises a detectable label attached to any suitable position, whereby said probe binds to an HCV polymerase or an analog thereof and is capable of being displaced by an inhibitor thereof.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DIRECT BINDING ASSAY FOR IDENTIFYING INHIBITORS OF HCV POLYMERASE

FIELD OF THE INVENTION

The present invention relates generally to a method for identifying inhibitors of the HCV RNA dependent RNA polymerase. Particularly, this method uses a novel probe in a competitive assay to identify HCV polymerase inhibitors and determine their potency. More particularly, this invention relates to the use of a probe which binds with specificity to the polymerase, and which is capable of being displaced by inhibitors of the enzyme.

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) is the major etiological agent of post-transfusion and community-acquired non-A non-B hepatitis worldwide. It is estimated that over 200 million people worldwide are infected by the virus. A high percentage of carriers become chronically infected and many progress to chronic liver disease, so called chronic hepatitis C. This group is in turn at high risk for serious liver disease such as liver cirrhosis, hepatocellular carcinoma and terminal liver disease leading to death. The mechanism by which HCV establishes viral persistence and causes a high rate of chronic liver disease has not been thoroughly elucidated. It is not known how HCV interacts with and evades the host immune system.

HCV is an enveloped positive strand RNA virus in the *Flaviviridae* family. The single strand HCV RNA genome is of positive polarity and comprises one open reading frame (ORF) of approximately 9600 nucleotides in length, which encodes a linear polyprotein of approx. 3010 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce structural and non-structural (NS) proteins. The structural proteins (C, E1, E2 and E2-p7) comprise polypeptides that constitute the virus particle (Hijikata, M. *et al.*, **1991**, Proc. Natl. Acad. Sci. USA. *88*, 5547-5551; Grakoui, A. *et al.*, **1993(a)**, J. Virol. *67*, 1385-1395). The non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B) encode for enzymes or accessory factors that catalyze and regulate the replication of the HCV RNA genome. Processing of the structural proteins is catalyzed by host cell proteases (Hijikata *et al.*, **1991**, *supra*). The generation of the mature non-structural proteins is catalyzed by two virally encoded proteases. The first is the NS2/3 zinc-

dependent metalloprotease which auto-catalyses the release of the NS3 protein from the polyprotein. The released NS3 contains a N-terminal serine protease domain (Grakoui A, *et al.*, **1993(b)**, Proc Natl Acad Sci USA, *90*, 10583-7; Hijikata, M. *et al.*, **1993**, J. Virol. *67*, 4665-4675.) and catalyzes the remaining cleavages from the polyprotein. The released NS4A protein has at least two roles. First, forming a stable complex with NS3 protein and assisting in the membrane localization of the NS3/NS4A complex (Kim *et al.*, Arch Virol. **1999,** 144: 329-343) and second, acting as a cofactor for NS3 protease activity. This membrane-associated complex, in turn catalyzes the cleavage of the remaining sites on the polyprotein, thus effecting the release of NS4B, NS5A and NS5B (Bartenschlager, R. *et al.*, **1993**, J. Virol., *67*, 3835-3844; Grakoui *et al.*, **1993(a)** *supra*; Hijikata *et al.*, **1993** *supra*; Love, R.A. *et al.*, **1996**, Cell, *87*, 331-342; reviewed in Kwong AD. *et al.*, **1998**, Antiviral Res., *40*, 1-18). The C-terminal segment of the NS3 protein also harbors nucleoside triphosphatase and RNA helicase activity (Kim, D.W. *et al.*, **1995**, Biochem. Biophys. Res. Comm., *215*, 160-166). The function of the protein NS4B is unknown. NS5A, a highly phosphorylated protein, seems to be responsible for the Interferon resistance of various HCV genotypes (Gale Jr. *et al.* **1997** Virology 230, 217; Reed *et al.*, **1997**, J. Virol. 71, 7187). NS5B is an RNA-dependent RNA polymerase (RdRp) that is involved in the replication of HCV.

To better understand the mechanism of HCV RNA replication and to develop appropriate *in vitro* systems, biochemical analyses of the NS5B protein have been performed. Full-length NS5B has been produced and purified as a non-fusion protein from insect cells infected with recombinant baculovirus (S.-E. Behrens *et al.*, **1996**, EMBO J., 15:12-22; R. de Francesco *et al.*, **1996**, Methods Enzymol., 275:58-67) or as a tagged protein from both insect cells (V. Lohmann *et al.*, **1997**, J. Virol., 71:8416-8428; V. Lohmann *et al.*, **1998**, Virology 249:108-118) and E. coli (Z.-H. Yuan *et al.*, **1997**, BBRC 232:231-235). *In vitro*, the RdRp activity of recombinant NS5B is dependent on an RNA template and requires RNA or DNA as a primer (S.-E. Behrens *et al,*, **1996**, EMBO J. 15:12-22; V. Lohmann *et al.*, **1997**, J. Virol., 71:8416-8428). On RNA templates of heteropolymeric sequences, the 3'-OH of the template is used as a primer and elongation proceeds via a "snap-back" mechanism, leading to a double-stranded molecule in which template and product RNA are covalently linked (S.-E. Behrens *et al.*, **1996**, EMBO J., 15:12-22; V. Lohman *et al.*, **1998**, Virology, 249:108-118; G. Luo *et al.*, **2000**, J. Virol. 74:851-863). Recently,

several groups also demonstrated that the HCV NS5B protein is able to initiate RNA synthesis *de novo* (J. Oh *et al.*, **1999**, J. Virol. 73:7694-7702; X. Sun *et al.*, **2000**, BBRC 268:798-803; W. Zhong *et al.*, **2000**, J. Virol. 74:2017-2022).

The NS5B RdRp has been crystallized to reveal a structure reminiscent of other nucleic acid polymerases (S. Bressanelli *et al.*, **1999**, PNAS USA 96:13034-13039; H. Ago *et al.*, **1999**, Structure 7:1417-1426; C.A. Lesburg *et al.*, **1999**, Nature Struct. Biol., 6:937-943). A comprehensive understanding of the differences between HCV and cellular polymerases will facilitate the design of specific inhibitors of HCV replication. Detailed kinetic information will also help in understanding the molecular basis of HCV NS5B-catalyzed nucleotide incorporation and subsequently the mechanistic characterization of the inhibitors.

Previous studies (S.-E. Behrens *et al.*, **1996**, EMBO J. 15:12-22; R. de Francesco *et al.*, **1996**, Methods Enzymol. 275:58-67; V. Lohmann *et al.*, **1997**, J. Virol. 71:8416-8428; V. Lohmann *et al.*, **1998**, Virology 249:108-118) provided little information with regard to the proportion of the polymerase RNA complexes that are competent for catalysis. Some recent studies investigated the template and primer requirements for HCV NS5B-directed RNA replication. Templates with 3'-termini free of secondary structures and short primers 2 or 3 nucleotides (nt) long were preferred for efficient initiation of RNA synthesis (W. Zhong *et al.*, **2000**, J. Virol. 74:9134-9143). In *de novo* initiation of RNA synthesis, however, NS5B needs a template with a stable secondary structure and a single-stranded sequence that contains at least one 3'-cytidylate.

Viral polymerases represent attractive targets for therapeutic inhibition of viral replication. The discovery of new antiviral agents often involves screening of large numbers of samples for inhibition of the target activity using either *in vitro* or *in vivo* assays. In general, polymerases are assayed by monitoring the incorporation of either ^{3}H-, α-^{32}P or α-^{33}P-labeled mononucleotides into oligonucleotide products, or by the extension of 5'-end-labeled primers. Products incorporated into the extended primers are captured or separated using common filter assays, acid precipitation, or denaturing gel electrophoresis.

The HCV NS5B polymerase is a prime target in the search for inhibitors of HCV

replication. Different preparations of the HCV polymerase exhibit varying efficiencies of product formation with a variety of RNA substrates. Moreover, the activity of purified recombinant NS5B polymerase varies significantly with specific RNA substrates. In addition, the *in vitro* RNA polymerase activity of NS5B is extremely sensitive to ionic strength, and salt concentrations exceeding 100 mM inhibit the reaction. Hence the ability to determine the potency of inhibitors at various salt concentrations is restricted by this limitation of standard enzymatic reactions. Also, HCV polymerase enzymatic assays disclosed in the prior art provide IC_{50} values as representative measurements of inhibitor potencies. For inhibitors that are competitive with either RNA or NTP, the IC_{50} value is proportional to the concentration of substrates in the assay and will vary depending on the concentration of these components.

In an effort to overcome the limitations of HCV polymerase assays that use sub-optimal and poorly characterized RNA substrates, the Applicants have developed an assay for identifying specific inhibitors of the HCV polymerase that is independent of RNA.

It is therefore an advantage of the present invention to provide an assay that permits a direct measurement of inhibitor potencies (reflected by Kd values as an unequivocal determination of inhibitor potency) under defined conditions, irrespective of the substrate concentration.

The direct binding assay of this invention is amenable to adjustments in salt concentration or pH levels beyond the restricted range required for RNA polymerization. This type of assay is amenable to a high sensitivity and a high throughput format.

It is a further advantage of the present invention to provide a probe that binds to the polymerase with a high affinity, and which is displaced by inhibitors of the enzyme.

It is a further advantage to provide an assay that is applicable to HCV polymerases of different genotypes.

SUMMARY OF THE INVENTION

In a first aspect of the invention, there is provided a method for identifying compounds binding to HCV polymerase comprising the steps of:

a) contacting said HCV polymerase or an analog thereof with a probe being capable of binding to an HCV polymerase or an analog thereof, said probe being displaceable by an inhibitor thereof, so as to form a complex comprising said probe bound to said polymerase;

b) measuring a signal emitted from said probe in said complex to establish a base line level;

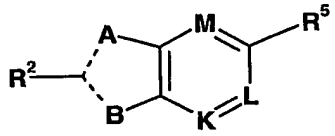
c) incubating the product of step a) with a test compound; and

d) measuring the signal from said complex; and

e) comparing the signal from step d) with the signal from step b);

whereby a modulation in said signal is an indication that said test compound binds to said polymerase.

In a preferred aspect the first embodiment, the probe is selected from: an isomer, enantiomer, diastereoisomer, or tautomer of a probe represented by formula I:



(I)

wherein:

A is O, S, N, NR^1, or CR^1, wherein R^1 is selected from the group consisting of: H, (C_{1-6})alkyl optionally substituted with:

-halogen, OR^{11}, SR^{11} or $N(R^{12})_2$, wherein R^{11} and each R^{12} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**, said aryl or **Het** optionally substituted with R^{10}; or

both R^{12} are covalently bonded together and to the nitrogen to which they are both attached to form a 5, 6 or 7-membered saturated heterocycle;

----- represents either a single or a double bond;

R^2 is selected from: H, halogen, R^{21}, OR^{21}, SR^{21}, $COOR^{21}$, $SO_2N(R^{22})_2$, $N(R^{22})_2$, , $CON(R^{22})_2$, $NR^{22}C(O)R^{22}$ or $NR^{22}C(O)NR^{22}$ wherein R^{21} and each R^{22} is independently H, (C_{1-6})alkyl, haloalkyl, (C_{2-6})alkenyl, (C_{3-7})cycloalkyl, (C_{2-6})alkynyl, $(C_{5-}$

$_{7}$)cycloalkenyl, 6 or 10-membered aryl or **Het**, said R^{21} and R^{22} being optionally substituted with R^{20};
or both R^{22} are bonded together to form a 5, 6 or 7-membered saturated heterocycle with the nitrogen to which they are attached;

B is NR^3 or CR^3, wherein R^3 is selected from (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl, (C_{6-10})bicycloalkyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**, said alkyl, cycloalkyl, bicycloalkyl, aryl, **Het**, alkyl-aryl and alkyl-**Het** being optionally substituted with from 1 to 4 substituents selected from: halogen, or

a) (C_{1-6})alkyl optionally substituted with:

- OR^{31} or SR^{31} wherein R^{31} is H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; or
- $N(R^{32})_2$ wherein each R^{32} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; or both R^{32} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

b) OR^{33} wherein R^{33} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**;

c) SR^{34} wherein R^{34} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; and

d) $N(R^{35})_2$ wherein each R^{35} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; or both R^{35} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

with the proviso that, when **A** is not N, then one of **A** or **B** is either CR^1 or CR^3;

K is N or CR^4, wherein R^4 is H, halogen, (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or R^4 is OR^{41} or SR^{41}, COR^{41} or $NR^{41}COR^{41}$ wherein each R^{41} is independently H, (C_{1-6})alkyl), (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl;
or R^4 is $NR^{42}R^{43}$ wherein R^{42} and R^{43} are each independently H, (C_{1-6})alkyl, $(C_{3-}$

$_7$)cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, or both R^{42} and R^{43} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

L is N or CR^5, wherein R^5 has the same definition as R^4 defined above;

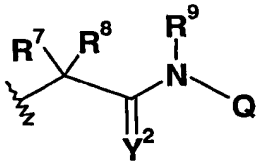
M is N or CR^7, wherein R^7 has the same definition as R^4 defined above;

R^5 is C(Y^1)-**Z** wherein Y^1 is O or S;

Z is N(R^{6a})R^6 or OR^6, wherein R^{6a} is H or (C_{1-6})alkyl or N$R^{61}R^{62}$ wherein R^{61} and R^{62} are each independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, or both R^{61} and R^{62} are covalently bonded together and to the nitrogen to which they are both attached to form a 5, 6 or 7-membered saturated heterocycle; or R^{62} is COOR^{63} wherein R^{63} is (C_{1-6})alkyl, (C_{3-6})cycloalkyl, said alkyl or cycloalkyl being optionally substituted with 6- or 10-membered aryl or **Het**; or R^{62} is COR^{64} wherein R^{64} is C_{1-6})alkyl, (C_{3-6})cycloalkyl -6-or 10-membered aryl or **Het**; and

R^6 is selected from the group consisting of: H, (C_{1-6})alkyl, (C_{3-6})cycloalkyl, (C_{2-6})alkenyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het**, wherein said alkyl, cycloalkyl, alkenyl, aryl, **Het**, alkyl-aryl, or alkyl-**Het**, are all optionally substituted with R^{60};

or R^6 is



wherein R^7 and R^8 are each independently H, (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het**, wherein said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het** are optionally substituted with R^{70};

or

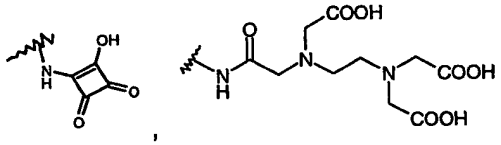
R^7 and R^8 are covalently bonded together to form second (C_{3-7})cycloalkyl or a 4, 5- or

6-membered heterocycle having from 1 to 4 heteroatom selected from O, N, and S; or when **Z** is $N(R^{6a})R^6$, either of R^7 or R^8 is covalently bonded to R^{6a} to form a nitrogen-containing 5-or 6-membered heterocycle;

Y^2 is O or S;

R^9 is H, (C_{1-6} alkyl), (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**, all of which optionally substituted with R^{90}; or
R^9 is covalently bonded to either of R^7 or R^8 to form a 5- or 6-membered heterocycle;

Q is a 6- or 10-membered aryl, **Het**, (C_{1-6}) alkyl-CONH-aryl or (C_{1-6}) alkyl-CONH-**Het**, all of which being optionally substituted with:



, or R^{100};

or a salt or a derivative thereof;

wherein **Het** is defined as 5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N, and S, or a 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N and S; and

R^{10}, R^{20}, R^{60}, R^{70}, R^{90} and R^{100} is each defined as:

- 1 to 4 substituents selected from: halogen, OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl,

Het, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H,

(C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150}; and

wherein R^{150} is defined as: - 1 to 3 substituents selected from: halogen, OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or

- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted

with R^{160};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SR^{161}, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the nitrogen to which they

are attached to form a 5, 6 or 7-membered saturated heterocycle,

wherein said probe comprises a detectable label attached to any suitable position, whereby said probe binds to an HCV polymerase or an analog thereof and is capable of being displaced by an inhibitor thereof.

According to an alternative of this first embodiment, the probe used for the assay does not comprise a detectable label, and the signal measured is the change in intrinsic fluorescence of the HCV polymerase in the presence and absence of said probe.

According to a second aspect of the invention, there is provided the use of a probe according to formula I in the development of an assay for identifying inhibitors of HCV polymerase.

According to a third aspect of the invention, there is provided a kit for testing compounds potentially binding to HCV polymerase, said kit comprising the probe of formula (I) and instructions on how to use said probe for identifying test compounds binding to said polymerase.

BRIEF DESCRIPTION OF THE FIGURES

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1 illustrates the titration of probe (i) with the NS5BΔ21-His polymerase. Standard conditions for the Fluorescence anisotropy analysis are described in Example 3. The determined K_d value of probe (i) for this polymerase is 12.6 nM.

Figure 2 illustrates Z' evaluation for the Fluorescence Polarization assay. A series of positive and negative controls were tested in the 96-well plate polarization assay, using the standard conditions, to determine the standard deviation (SD) of both controls. The Z' value was then obtained from the following calculation :

$$Z' = 1 - \frac{(3\ SD\ of\ pos.\ ctrls + 3\ SD\ of\ neg.\ ctrls)}{(mean\ pos.\ ctrl - mean\ neg.\ ctrl)}$$

Figure 3 illustrates K_d determination for Compounds A and B, using the Fluorescence Polarization assay. Standard conditions of the 96-well plate Polarization assay (see Example 4) were used to determine the K_d values of the compounds. K_d values obtained for compound A and B are 31 and 41 nM, respectively, with Q_b/Q_f values of 0.67 and 0.72.

Figure 4 illustrates K_d determination for Compounds C and D, using the Fluorescence Polarization assay. Standard conditions of the 96-well plate Polarization assay (see Example 4) have been used to determine the K_d values of some of our compounds. K_d values obtained for compound C and D are 231 nM and 1.08 uM, respectively, with Q_b/Q_f values of 0.74 and 0.66.

Figures 5 to 8 illustrate the titration of probe (i) with the NS5BΔ21-His in the presence of increasing (from 30 mM to 200 mM) concentration of NaCl. Standard conditions of the Fluorescence anisotropy analysis are described in Example 3. K_d values obtained for this polymerase are 15.3 nM (30 mM NaCl), 39 nM (100 mM NaCl), 78 nM (150 mM NaCl) and finally 122 nM (200 mM NaCl).

Figure 9 illustrates the titration of probe (i) with the NS5BΔ21-His in Phosphate buffer pH 6.5. Standard conditions of the Fluorescence anisotropy analysis are described in Example 3. The K_d of probe (i) for this polymerase under these conditions is 33 nM.

Figure 10 illustrates the titration of probe (i) with the His-NS5BΔ21 polymerase. Standard conditions of the Fluorescence anisotropy analysis are described in Example 3. The K_d of probe (i) for this N-terminally tagged polymerase is 18.1 nM.

Figure 11 illustrates the titration of probe (ii) with the GBV-BΔ23-His polymerase. Standard conditions of the Fluorescence anisotropy analysis are described in Example 3. The K_d of probe (ii) for this distantly related polymerase is 1.79 uM (estimated value with an incomplete curve).

Figure 12 illustrates the titration of probe (ii) with the His-NS5BΔ21(H77c, HCV genotype 1a) polymerase. Standard conditions of the Fluorescence anisotropy analysis are described in Example 3. The K_d of probe (ii) for this HCV genotype 1a polymerase is 18.2 nM.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

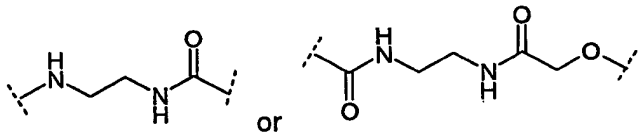
The following definitions apply unless otherwise noted:

The term "affinity tag" means a moiety whose strong affinity for a ligand can be used to extract from a solution the entity to which the tag is attached. Examples of such tags include biotin or a derivative thereof, a histidine polypeptide, a polyarginine, an amylose sugar moiety or a defined epitope recognizable by a specific antibody. Such affinity tags are attached to the probe by well-known methods. The corresponding affinity ligands are also well known in the art.

An "analog" of the HCV NS5B polypeptide or a fragment thereof means a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such analogs of the natural amino acid sequence may involve insertion, addition, deletion or substitution of one or more amino acids, and may or may not alter the functional activity of the original HCV NS5B polypeptide. As mentioned above, the HCV NS5B polypeptide or protein used in the assay/method of the invention includes any fragment, derivative, variant or mutant which is derived from a HCV NS5B polypeptide and which retains at least one property or other characteristic of the HCV NS5B polypeptide.

The term "detectable label" refers to any group that is linked to a probe of the present invention such that when the probe is associated with the polymerase target, such label allows recognition either directly or indirectly of the probe such that it can be detected, measured and quantified. Examples of such "detectable labels" are intended to include, but are not limited to: photoreactive groups, fluorescent labels, chemiluminescent labels, colorimetric labels, enzymatic markers, radioactive isotopes. Such labels are attached to the probe by well known methods.

As used herein, the term "linker" refers to a chain of between 1 and 20 atoms selected from the group consisting of C, N, O, and S that covalently connects the aforesaid label to a probe of the present invention. Examples of such a chain include, but are not limited to, the following:



These linkers can also comprise a pair of affinity-tag/affinity-ligand, which together, bind the compound to a detectable label.

The term "photoreactive group" means a group that is transformed, upon activation by light, from an inert group to a reactive species, such as a free radical. Examples of such groups include, but are not limited to, benzophenones, azides, and the like.

As used herein, the term "probe" refer to a compound of formula (I) that is capable of binding to an HCV polymerase in a covalent or non-covalent manner. When the probe is bound in a non-covalent manner, it can be displaced by a test compounds. When bound in a covalent manner, the probe can be used for cross-linking experiments wherein the HCV polymerase-probe adduct formation can be quantified and inhibited by test compounds.

As used herein, the terms "(C_{1-3}) alkyl", "(C_{1-4}) alkyl" or "(C_{1-6}) alkyl", either alone or in combination with another radical, are intended to mean acyclic straight or branched chain alkyl radicals containing up to three, four and six carbon atoms respectively. Examples of such radicals include methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl.

As used herein, the term "(C_{2-6}) alkenyl", either alone or in combination with another radical, is intended to mean an unsaturated, acyclic straight chain radical containing two to six carbon atoms.

As used herein, the term (C_{2-6}) alkynyl" either alone or in combination with another group, is intended to mean an unsaturated, acyclic straight chain sp hybridized radical containing 2 to six carbon atoms.

As used herein, the term "(C_{3-7}) cycloalkyl", either alone or in combination with another radical, means a cycloalkyl radical containing from three to seven carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

As used herein, the term "(C_{5-7})cycloalkenyl", either alone or in combination with another radical, means an unsaturated cyclic radical containing five to seven carbon atoms.

As used herein, the term "aryl", or "6- or 10-membered aryl" either alone or in combination with another radical means aromatic radical containing six or ten carbon atoms, for example phenyl or naphthyl.

As used herein, the term "COOH" refers to a carboxylic acid group. It is well known to one skilled in the art that carboxylic acid groups may be substituted by functional group equivalents. Examples of such functional group equivalents that are contemplated by this invention include, but are not limited to, esters, amides, or boronic acids.

As used herein, the term "functional group equivalent" is intended to mean an element or a substituted derivative thereof, that is replaceable by another element that has similar electronic, hybridization or bonding properties.

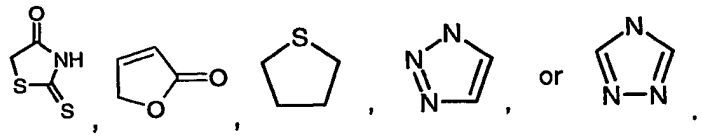
As used herein, the term "halo" means a halogen atom and includes fluorine, chlorine, bromine and iodine.

As used herein, the term "haloalkyl" is intended to mean an alkyl that is described above in which each hydrogen atom may be successively replaced by a halogen atom, for example CH_2Br or CF_3.

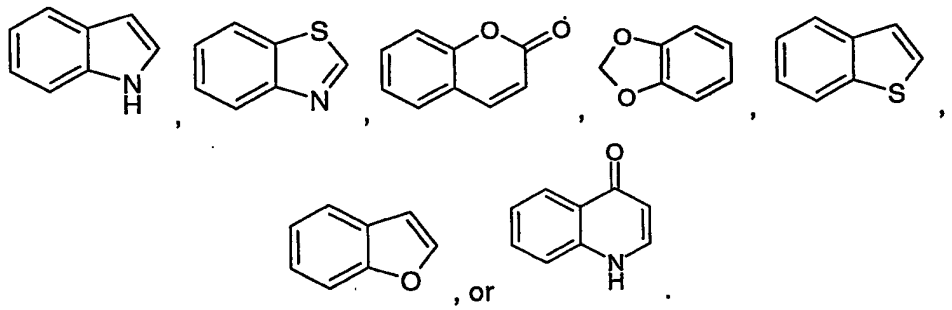
As used herein the term heteroatom means O, S or N.

As used herein, the term "heterocycle", either alone or in combination with another radical, means a monovalent radical derived by removal of a hydrogen from a five-, six-, or seven-membered saturated or unsaturated (including aromatic) heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur.

Furthermore, "heterobicyclic" as used herein, means a heterocycle as defined above fused to one or more other cycle, be it a heterocycle or any other cycle. Examples of such heterocycles include, but are not limited to, pyrrolidine, tetrahydrofuran, thiazolidine, pyrrole, thiophene, coumarin, hydantoin, diazepine, 1H-imidazole, isoxazole, thiazole, tetrazole, piperidine, 1,4-dioxane, 4-morpholine, pyridine, pyridine-N-oxide, pyrimidine, thiazolo[4,5-b]-pyridine, quinoline, or indole, or the following heterocycles:



As used herein, the term "9- or 10-membered heterobicycle" or "heterobicycle" either alone or in combination with another radical, means a heterocycle as defined above fused to one or more other cycle, be it a heterocycle or any other cycle. Examples of such heterobicycles include, but are not limited to, thiazolo[4,5-b]-pyridine, quinoline, or indole, or the following:



As used herein, the term "**Het**" defines a 5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N, and S, or a 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N and S.

As used herein, the term "OH" refers to a hydroxyl group. It is well known to one skilled in the art that hydroxyl groups may be substituted by functional group equivalents. Examples of such functional group equivalents that are contemplated by this invention include, but are not limited to, ethers, sulfhydryls, and primary, secondary or tertiary amines.

As used herein, the term "SH" refers to a sulfhydryl group. It is intended within the scope of the present invention that , whenever a "SH" or "SR" group is present, it can also be substituted by any other appropriate oxidation state such as SOR, SO_2R, or SO_3R.

It is intended that the term "substituted" when applied in conjunction with a radical having more than one moiety such as C_{1-6}alkyl-aryl, or C_{1-6}alkyl-**Het**, such substitution applies to both moieties i.e. both the alkyl and aryl or **Het** moieties can be substituted with the defined substituents.

Description of preferred embodiments

Preferably, according to the first aspect, the invention provide a method for identifying inhibitors of HCV polymerase comprising the steps of:

a) contacting said HCV polymerase with a probe of formula I so as to form a complex comprising said probe bound to said polymerase;

b) measuring a signal from said complex to establish a base line level;

c) incubating the product of step a) with a test compound;

d) measuring the signal from said complex; and

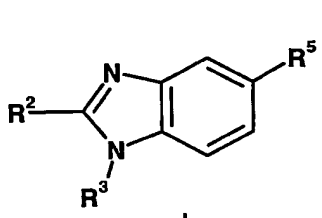
e) comparing the signal from step d) with the signal from step b);

whereby a decrease in said signal is an indication that said test compound is an inhibitor of said polymerase.

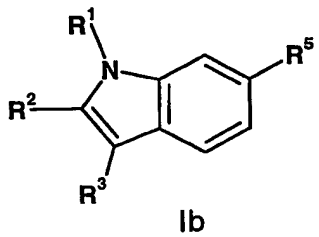
As will be understood by a person skilled in the art, the association of a specific probe of the invention with the NS5B polymerase can be measured directly or indirectly in a variety of ways. The probe and NS5B polymerase need not be labeled and affinity tagged respectively. The association of a specific probe with the HCV NS5B polymerase can be monitored and quantified directly by a change in the intrinsic spectral properties of a tagged or un-tagged NS5B protein and/or by a change in the intrinsic spectral properties of a specific probe. A direct measurement of inhibitor-NS5B association can also be achieved by immobilizing one of these two components on a matrix and measuring association through plasma-resonance detection technology. An assay that quantifies probe-NS5B complex association may also incorporate a photo-reactive label (such as a phenyl-azide or benzophenone) on the probe (for example probes (v) and (vi) below) and measure the amount of

label irreversibly bound to the NS5B (adduct) following photo-activation of the probe.

Preferably, according to a first aspect of the present invention, there is provided a probe of formula:

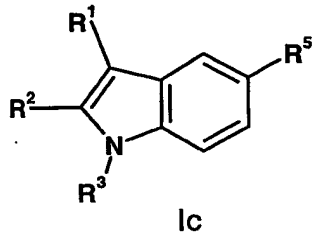


Ia



Ib

or



Ic

wherein

R^1 is selected from the group consisting of: H or (C_{1-6})alkyl;

R^2 is $CON(R^{22})_2$, wherein each R^{22} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{5-7})cycloalkenyl, 6 or 10-membered aryl or **Het**, or both R^{22} are bonded together to form a 5, 6 or 7-membered saturated heterocycle with the nitrogen to which they are attached;

or R^2 is selected from: H, halogen, (C_{1-6})alkyl, haloalkyl, (C_{2-6})alkenyl, (C_{5-7})cycloalkenyl, 6 or 10-membered aryl or **Het**; wherein each of said alkyl, haloalkyl, (C_{2-6})alkenyl, (C_{5-7})cycloalkenyl, aryl or **Het** is optionally substituted with R^{20}, wherein R^{20} is defined as:

- 1 to 4 substituents selected from: halogen, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl,

Het, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150}; or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated

heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with $\mathbf{R^{150}}$;

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with $\mathbf{R^{150}}$;

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with $\mathbf{R^{150}}$;

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with $\mathbf{R^{150}}$;

l) aryl, **Het**, (C1-6alkyl)aryl or (C1-6alkyl)**Het**, all of which being optionally substituted with $\mathbf{R^{150}}$;

wherein $\mathbf{R^{150}}$ is preferably:

- 1 to 3 substituents selected from: halogen, NO_2, cyano or azido; or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with $\mathbf{R^{160}}$;

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl) or (C_{3-7})cycloalkyl, said alkyl or cycloalkyl optionally substituted with $\mathbf{R^{160}}$;

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het** and heterocycle being optionally substituted with $\mathbf{R^{160}}$;

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, or (C_{3-7})cycloalkyl, and R^{112} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to

form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl said (C_{1-6})alkyl and (C_{3-7})cycloalkyl being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

said alkyl, cycloalkyl, and heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl and cycloalkyl being optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl) or (C_{3-7})cycloalkyl, or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl and cycloalkyl being optionally substituted with R^{160};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said (C_{1-6})alkyl and (C_{3-7})cycloalkyl being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is

independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

R^3 is selected from (C_{3-7})cycloalkyl, (C_{6-10})bicycloalkyl, 6- or 10-membered aryl, or **Het**;

R^5 is -C(O)-**Z**, wherein

Z is OR^6 wherein R^6 is C_{1-6}alkyl substituted with:

- 1 to 4 substituents selected from: OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is $(C_{1-6}$alkyl) substituted with R^{150}, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, and R^{112} is CN, (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-$(C_{3-}$

$_7$)cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-}

$_6$alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150};

- 1 to 3 substituents selected from: halogen, NO_2, cyano, azido or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the

nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or **Z** is $N(R^{6a})R^6$, wherein R^{6a} is H or (C_{1-6}alkyl); and

R^6 is (C_{1-6})alkyl optionally substituted with:

- 1 to 4 substituents selected from: OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl substituted with R^{150a}, haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150}, wherein R^{150a} is the same as R^{150} but is not halogen, OR^{150b}, $COOR^{150b}$, $N(R^{150b})_2$, wherein R^{150b} is H or C_{1-6}alkyl;

b) OR^{104} wherein R^{104} is (C_{1-6}alkyl) substituted with R^{150}, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** or
R^{111} is H and R^{112} is SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both

R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-}

$_{7}$)cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150}; and

wherein R^{150} is selected from:

- 1 to 3 substituents selected from: halogen, NO_2, cyano, azido or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

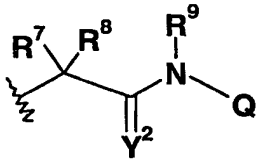
j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from:

tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle.

or R^6 is



wherein, preferably, R^7 and R^8 are each independently H, (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het**, wherein said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het** are optionally substituted with R^{70}; or

R^7 and R^8 are covalently bonded together to form second (C_{3-7})cycloalkyl or a 4, 5- or 6-membered heterocycle having from 1 to 3 heteroatom selected from O, N, and S; or when **Z** is $N(R^{6a})R^6$, either of R^7 or R^8 is covalently bonded to R^{6a} to form a nitrogen-containing 5-or 6-membered heterocycle;

wherein, preferably, R^{70} is selected from:

- 1 to 4 substituents selected from: halogen, NO_2, cyano, azido; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

d) $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or both R^{108} are covalently bonded together and to

the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
and R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, $(C_{1-6}$alkyl)aryl or (C1-6alkyl)**Het**, all of which being optionally substituted with R^{150};

wherein, preferably, R^{150} is selected from:

- 1 to 3 substituents selected from: halogen, NO_2, cyano, azido; or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, $(C_{1-6}$alkyl) or (C_{3-7})cycloalkyl, said alkyl and cycloalkyl being optionally substituted with R^{160};

d) $SO_2N(R^{108})_2$ wherein R^{108} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl or cycloalkyl being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, and R^{112} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het**, $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said (C_{1-6})alkyl or (C_{3-7})cycloalkyl being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl; or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl or cycloalkyl being optionally substituted with R^{160}; or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl) or (C_{3-7})cycloalkyl, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

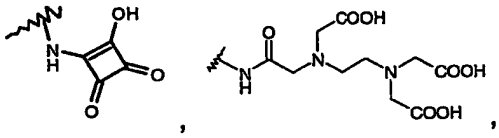
j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said (C_{1-6})alkyl and (C_{3-7})cycloalkyl being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, OR^{161}, $N(R^{162})_2$ or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H or (C_{1-6})alkyl;

R^9 is H; or R^9 is covalently bonded to either of R^7 or R^8 to form a 5- or 6-membered heterocycle; and

Q is a 6- or 10-membered aryl, **Het**, all of which being optionally substituted with:


, or R^{100};

wherein R^{100} is:

- 1 to 4 substituents selected from: halogen, NO_2, cyano or azido; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-}

$_7$)cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150};

wherein R^{150} is selected from:

- 1 to 3 substituents selected from: halogen, NO_2, cyano or azido; or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-6})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are

covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-}

$_7$)cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{160};

j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{160}; and

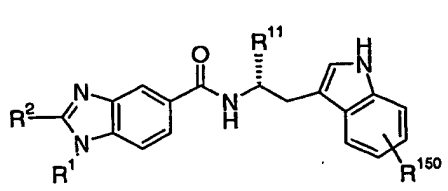
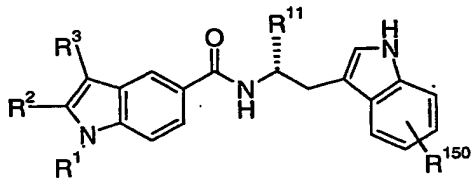
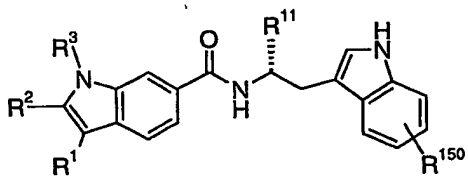
k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SR^{161}, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or a salt thereof;

wherein said probe comprises a detectable label attached to any suitable position, whereby said probe binds to an HCV polymerase or an analog thereof and is capable of being displaced by an inhibitor thereof.

More preferably, the probe of the invention is a compound of formula:


(IIa),

(IIb),

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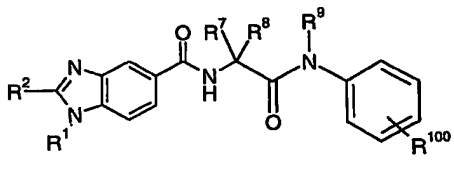
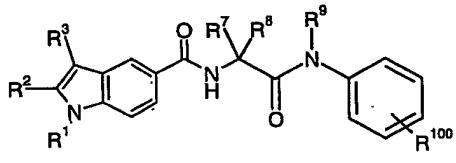
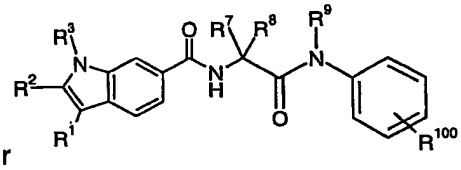

(IId)

(IIe) or

(IIf).

wherein R^1 is (C_{5-6})cycloalkyl;

R^2 is phenyl, or **Het** both being optionally substituted with R^{20};

R^3, R^7, R^8 ,R^9, R^{100}, and R^{150} are as defined above;

R^{11} is OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or

a) (C_{1-6}) alkyl substituted with R^{150a}, haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150}, wherein R^{150a} is the same as R^{150} but is not halogen, OR^{150b}, $COOR^{150b}$, $N(R^{150b})_2$, wherein R^{150b} is H or C_{1-6}alkyl;

b) OR^{104} wherein R^{104} is (C_{1-6}alkyl) substituted with R^{150}, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl,

Het, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** or

R^{111} is H and R^{112} is SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are

covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with $\mathbf{R^{150}}$;

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with $\mathbf{R^{150}}$;

j) $COOR^{128}$ wherein R^{128} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150}

wherein R^{150} is as defined herein;

or a salt thereof;

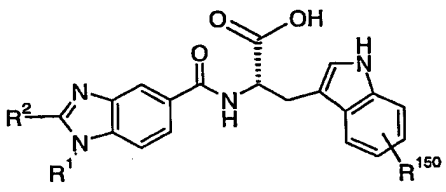
wherein said compound is either:

a) marked with a radioactive isotope at any suitable position;

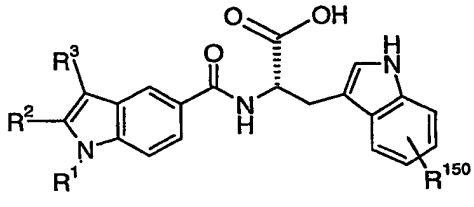
b) linked to a detectable moiety by a suitable linker at any suitable position, except R^1 and R^3; or

c) linked to an affinity tag at any suitable position, except R^1 and R^3.

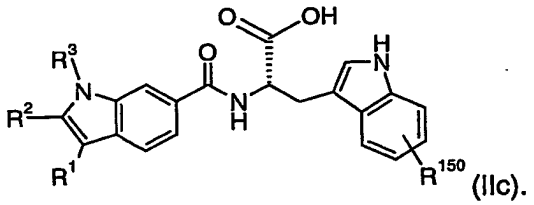
Even more preferably, the probe of the invention is a compound of formula:



(IIa),



(IIb),



(IIc).

wherein

R^1 is (C_{5-6})cycloalkyl;

R^2 is phenyl, or **Het** both being optionally substituted with R^{20};

R^3 and R^{150} are as defined above;

or a salt thereof;

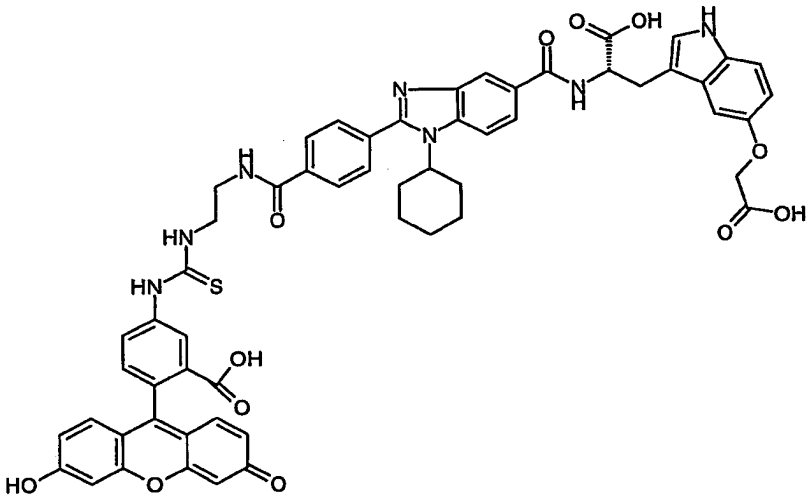
wherein said compound is optionally:

a) marked with a radioactive isotope at any suitable position;

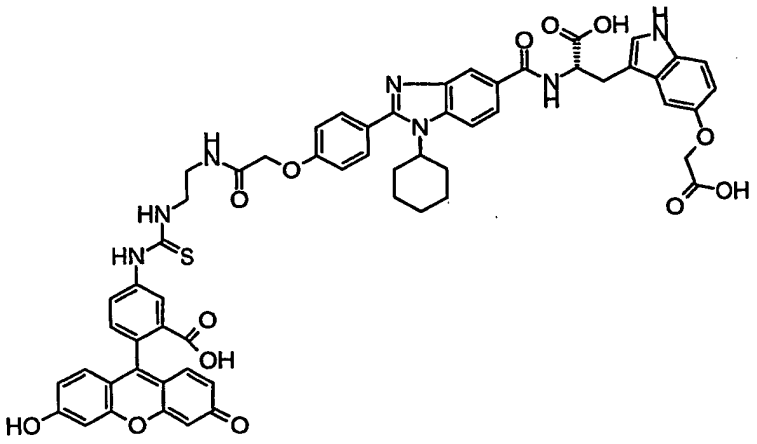
b) linked to a detectable moiety by a suitable linker at any suitable position, except R^1 and R^3; or

c) linked to an affinity tag at any suitable position, except R^1 and R^3.

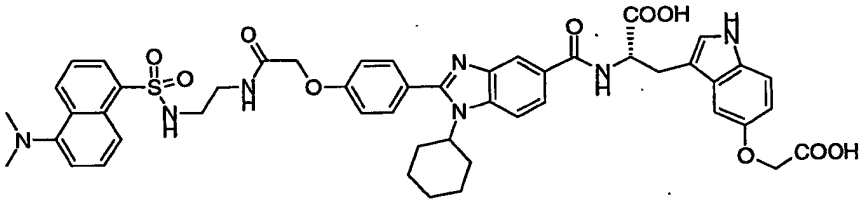
Specifically, according to a first aspect of the invention, the probe of the present invention is selected from the group consisting of:



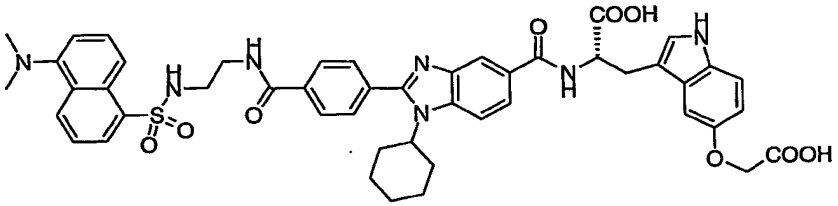
(i);



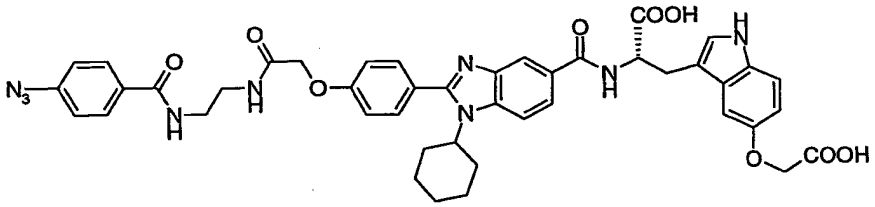
(ii);



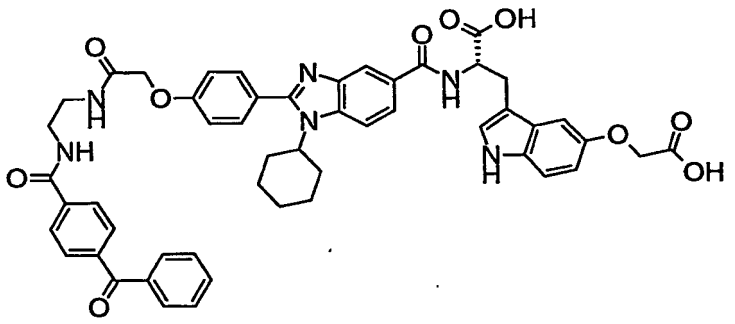
(iii);



(iv);



(v); and



(vi)

According to an alternative aspect of this first embodiment, there is provided a method for identifying compounds that inhibit HCV polymerase comprising the steps of:

a) contacting said HCV polymerase or an analog thereof with a probe of formula I, as defined herein, so as to form a complex having said probe bound to said polymerase;

b) measuring the signal from said complex to establish a base line level;

c) incubating the product of step a) with a test compound; and

d) measuring the signal from said complex; and

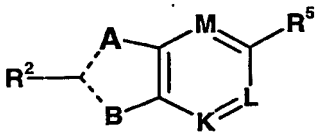
e) comparing the signal from step d) with the signal from step b);

whereby a modulation in said signal is an indication that said test compound inhibits said polymerase.

Preferably, the method for identifying compounds capable of inhibiting HCV polymerase, comprises:

f) repeating steps (a) to (e), as defined above in a high throughput screen.

Alternatively, there is provided a probe of formula I:



I

A is O, S, NR^3, or CR^3;

B is NR^1 or CR^1; with the proviso that, when **A** is CR^3, **B** is NR^1, and when **A** is O or S, **B** is CR^1;

----- represents either a single or a double bond;

R^1 is selected from the group consisting of: (C_{4-7})cycloalkyl optionally substituted with (C_{1-6} alkyl); norbornane, 5-, 6- or 7-membered heterocycle having 1 to 4 heteroatoms selected from O, N, and S, all of which optionally substituted with 1 to 4 substituent selected from the group consisting of:

halo, OH and C_{1-6} alkyl optionally substituted with hydroxy;

R^2 is selected from the group consisting of: phenyl, pyridine-N-oxide, 5- or 6-membered aromatic heterocycle having 1 to 4 heteroatoms selected from O, N, and S, and 9- or 10-membered aromatic heterobicycle having 1 to 4 heteroatoms selected from O, N and S;

said phenyl, pyridine-N-oxide, aromatic heterocycle and aromatic heterobicycle being optionally substituted with from 1 to 4 substituents selected from the group consisting of: halogen, C_{1-6} haloalkyl, (C_{1-6})alkyl, C_{1-6} alkoxy, OH, amino optionally mono- or di-substituted with C_{1-6} alkyl;

R^3 is selected from the group consisting of: H, (C_{1-6})alkyl, (C_{1-6} alkyl)-(C_{6-10}aryl), (C_{1-6} alkyl)-5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N, and S, and 5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N and S,

wherein said aryl and said heterocycle are optionally substituted with from 1 to 4 substituents selected from the group consisting of: COOH, COO(C_{1-6} alkyl), halogen, and (C_{1-6} alkyl);

M is N, CR^{4a}, or COR^{4b}, wherein R^{4a} is selected from the group consisting of: H, halogen, and (C_{1-6} alkyl); and R^{4b} is selected from the group consisting of: H and (C_{1-6} alkyl);

K and **L** is each independently N or CR^6, wherein R^6 is H, halo, C_{1-6} alkyl, OH, or C_{1-6} alkoxy;

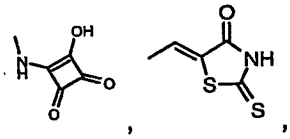
R^5 is -C(**Y**)-Z, wherein **Y** is O or S; and **Z** is NHR^{5a} or OR^{5a};

wherein:

R^{5a} is selected from the group consisting of: H, (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{3-6})cycloalkyl optionally substituted with C_{1-6}alkyl or C_{2-6}alkenyl, (C_{6-10})aryl optionally substituted with C_{1-6}alkyl or C_{2-6}alkenyl, N{(C_{1-6}) alkyl}$_2$, NHCOO(C_{1-6})alkyl(C_{6-10})aryl, NHCO(C_{6-10})aryl, -5- or 6-atom heterocycle, having 1 to 4 heteroatoms selected from O, N and S, and -9- or 10-atom heterobicycle having 1 to 4 heteroatoms selected from O, N and S;

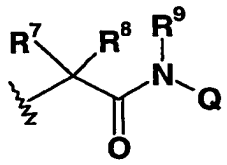
wherein said alkyl, alkenyl, cycloalkyl, aryl, heterocycle or heterobicycle are all optionally substituted with from 1 to 4 substituents selected from: OH, COOH, (C_{1-6})alkyl, (C_{2-4})alkenyl, (C_{1-6})alkyl-hydroxy, $COO(C_{1-6})$alkyl, C_{3-7} cycloalkyl, benzyloxy, halogen, (C_{2-4})alkenyl-(C_{1-6})alkyl-COOH, coumarin, (C_{1-6})alkyl-amino, $NH(C_{1-6}$ alkyl), $C(halogen)_3$, $-C(O)NH(C_{1-4})$alkyl, and $-C(O)NH(C_{6-10})$aryl, 5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N and S, 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N and S, and 6- or 10-membered aryl;

wherein said alkyl, alkenyl, cycloalkyl, aryl, heterocycle and heterobicycle are all optionally substituted with from 1 to 4 substituents selected from: halogen, OPO_3H, sulfonamido, SO_3H, SO_2CH_3, $-CONH_2$, $-COCH_3$, (C_{1-3})alkyl, $(C_{2-4}$alkenyl)COOH, tetrazolyl, COOH, $-CONH_2$, triazolyl, OH, NO_2, NH_2, $-O(C_{1-6}$ alkyl)COOH, hydantoin, benzoyleneurea, (C_{1-4})alkoxy, cyano, azido, -O-(C_{1-6})alkyl COOH, -O-(C_{1-6})alkyl COO-(C_{1-6})alkyl, NHCO-$(C_{1-6}$alkyl), -NHCOCOOH, $-NHCOCONHOH$,$-NHCOCONH_2$, $-NHCOCONHCH_3$, $-NHCO(C_{1-6})$alkyl-COOH, $-NHCOCONH(C_{1-6})$alkyl-COOH, $-NHCO(C_{3-7})$cycloalkyl-COOH, $-NHCONH(C_{6-10})$aryl-COOH, $-NHCONH(C_{6-10})$aryl-$COO(C_{1-6})$alkyl, - $NHCONH(C_{1-6})$alkyl-COOH,- $NHCONH(C_{1-6})$ alkyl-$COO(C_{1-6})$alkyl, - $NHCONH(C_{1-6})$alkyl-(C_{2-6})alkenyl-COOH, - $NH(C_{1-6})$alkyl-(C_{6-10})aryl-$O(C_{1-6})$alkyl COOH, - $NH(C_{1-6})$alkyl-(C_{6-10})aryl-COOH, $-NHCH_2COOH$, $-NHCONH_2$, $-NHCO(C_{1-6})$hydroxyalkyl COOH, $-OCO(C_{1-6})$hydroxyalkyl COOH, (C_{3-6})cycloalkyl COOH,



, -NHCN, -NHCHO, $-NHSO_2CH_3$, $-NHSO_2CF_3$; and $-O(C_{1-6}$alkyl)-tetrazol;

or R^{5a} is



wherein R^7 and R^8 are each independently H, (C_{1-6} alkyl), (C_{3-7} cycloalkyl), (C_{1-6} alkyl)phenyl, (C_{1-6} alkyl)-(C_{3-7} cycloalkyl), (C_{3-7} cycloalkyl)-(C_{1-6} alkyl), (C_{3-7} cycloalkyl)-(C_{2-4} alkenyl), (C_{1-6} alkyl)-OH, phenyl, CH_2biphenyl, 5- or 6-membered heterocycle having from1 to 4 heteroatoms selected from O, N, and S, 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N, and S, (C_{1-6} alkyl)-5- or 6-membered heterocycle having from1 to 4 heteroatoms selected from O, N, and S, or (C_{1-6} alkyl)-9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N, and S,

or R^7 and R^8 are covalently bonded together to form (C_{3-7} cycloalkyl), 4-, 5- or 6-membered heterocycle having from1 to 4 heteroatoms selected from O, N, and S; or one of R^7 or R^8 is covalently bonded to R^9 to form a pyrrolidine;

wherein said alkyl, cycloalkyl, heterocycle, heterobicycle, phenyl are optionally substituted with from 1 to 4 substituents selected from the group consisting of: OH, COOH, (C_{1-6} alkyl), (C_{2-4} alkenyl), $CONH_2$, NH_2, $NH(C_{1-6}$ alkyl), $N(C_{1-6}$ alkyl)$_2$, NHCOCOOH, $NHCOCON(C_{1-6}$ alkyl)$_2$, $NHCOCONH(C_{1-6}$ alkyl), SH, $S(C_{1-6}$ alkyl), $NHC(=NH)NH_2$, halogen, and $COO(C_{1-6}$alkyl);

R^9 is H or (C_{1-6} alkyl); and

Q is selected from the group consisting of: (C_{1-3}alkyl)CONHaryl, 6- or 10-membered aryl, biphenyl, 5- or 6-atom heterocycle having 1 to 4 heteroatoms selected from O, N and S, 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N and S;

wherein said aryl, biphenyl, heterocycle and heterobicycle are all optionally substituted with from 1 to 4 substituents selected from: OH, COOH, $COO(C_{1-6})$alkyl, (C_{1-6})alkyl, (C_{1-6})alkylCOOH, (C_{1-6} alkyl)(C_{2-4} alkynyl), (C_{1-6})alkyl-hydroxy, phenyl, benzyloxy, halogen, (C_{2-4})alkenyl, (C_{2-4})alkenyl-(C_{1-6})alkyl-COOH, 5- or 6-membered second heterocycle having 1 to 4 heteroatoms selected from O, N and S, NH-5- or 6- membered second heterocycle having 1 to 4 heteroatoms selected from O, N, and S,

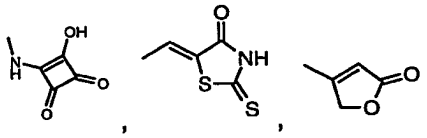
wherein said second heterocycle and phenyl being optionally substituted with from 1 to 4 substituents selected from: (C_{1-6} alkyl), CF_3, OH, (C_{1-6}alkyl) COOH, $O(C_{1-6}$alkyl)COOH, (C_{1-6}alkyl) $COO(C_{1-}$

$_6$alkyl), CH_2phenyl, $COO(C_{1-6}$ alkyl), (C_{1-6}alkyl)$O(C_{1-6}$alkyl), COOH, $NCH(C_{1-6}alkyl)_2$, $NCO(C_{1-6}$ alkyl), NH_2, $NH(C_{1-6}$ alkyl), halogen, $N(C_{1-6}$ alkyl)$_2$; and C_{2-6} alkenyl-COOH

halogen, OPO_3H, benzyl, sulfonamido, SH, $SOCH_3$, SO_3H, SO_2CH_3, $S(C_{1-6}$ alkyl)COOH, $-CONH_2$, $-COCH_3$, (C_{1-3})alkyl, $(C_{2-4}$alkenyl)COOH

wherein said alkenyl is optionally substituted with from 1 to 2 (C_{1-6} alkyl) substituents,

$(C_{2-4}$alkenyl)$COO(C_{1-6}$alkyl), tetrazolyl, COOH, triazolyl, OH, NO_2, NH_2, , -$O(C_{1-6}$ alkyl)COOH, hydantoin, benzoyleneurea, (C_{1-4})alkoxy, (C_{1-4})alkoxy(C_{1-6} alkyl)COOH, cyano, azido, -O-(C_{1-6})alkyl COOH, -O-(C_{1-6})alkyl COO-(C_{1-6})alkyl, -NHCOCOOH, -NHCOCONHOH,-$NHCOCONH_2$, $-NHCOCONHCH_3$, -NHCO(C_{1-6})alkyl-COOH, -NHCOCONH(C_{1-6})alkyl-COOH, -NHCO(C_{3-7})cycloalkyl-COOH, -NHCONH(C_{6-10})aryl-COOH, - NHCONH(C_{6-10})aryl-COO(C_{1-6})alkyl, - NHCONH(C_{1-6})alkyl-COOH,- NHCONH(C_{1-6})alkyl-COO(C_{1-6})alkyl, - NHCONH(C_{1-6})alkyl-(C_{2-6})alkenyl-COOH, - NH(C_{1-6})alkyl-(C_{6-10})aryl-O(C_{1-6})alkyl COOH, - NH(C_{1-6})alkyl-(C_{6-10})aryl-COOH, $-NHCH_2COOH$, $-NHCONH_2$, -NHCO(C_{1-6})hydroxyalkyl COOH, -OCO(C_{1-6})hydroxyalkyl COOH, (C_{3-6})cycloalkyl COOH,



, -NHCN, -NHCHO, $-NHSO_2CH_3$, $-NHSO_2CF_3$, coumarin, (C_{1-6})alkyl-amino, $NH(C_{1-6}alkyl)_2$, C(halogen)$_3$, -NH(C_{2-4})acyl, -NH(C_{6-10})aroyl, -CONH$(C_{1-6}$alkyl), -CO(C_{1-6})alkyl-COOH, -CONH(C_{1-6})alkyl-COOH, -CO-NH-alanyl, -CONH(C_{2-4})alkylN$(C_{1-6}$alkyl)$_2$, -CONH(C_{2-4}) alkyl-Het, -CONH(C_{2-4}) alkyl-(COOH)-Het, -CONH$(C_{1-2}$ alkyl)(OH)$(C_{1-2}$ alkyl)OH, -CONH(C_{1-6}) alkyl-COOH, -CONH$(C_{6-10}$ aryl), -CONH-Het, -CONH(C_{6-10}) aryl-COOH, -CONH(C_{6-10}) aryl-COO(C_{1-6}) alkyl, -CONH(C_{1-6}) alkyl-COO(C_{1-6}) alkyl, -CONH(C_{6-10}) aryl-(C_{1-6})alkyl-COOH, and -CONH(C_{6-10}) aryl-(C_{2-6})alkenyl-COOH;

or a salt thereof;

said probe comprises a detectable label, whereby said probe binds to an HCV polymerase or an analog thereof and is capable of being displaced by an inhibitor thereof.

Labels incorporated into the probe may be paired with appropriate labels attached to the tagged NS5B polymerase such that the close proximity of the two pairs of labels upon probe–polymerase association results in a measurable signal; examples of such detection techniques include, but are not limited to, fluorescence resonance energy transfer (FRET), and time resolved fluorescence (TRF).

Preferably, the detectable label is selected from the group consisting of: a fluorescent label (such as fluorescein, Oregon green, dansyl, rhodamine, Texas-red, phycoerythrin or $Eu^{3=}$), a radioactive atom (such as ^{3}H, ^{14}C, ^{125}I), a chemiluminescent label (such as luciferase), colorimetric produced by an enzymatic marker (such as β-galactosidase or horseradish peroxidase).

Alternatively, a fluorescent reporter and quencher may be used as pair of labels to monitor association of the probe with the HCV NS5B polymerase. Commonly known reporter/quencher pair may be selected from, for example: EDANS/DABCYL, tryptophan/2,4-dinitrophenyl, tryptophan/DANSYL, 7-methoxycoumarin/2,4-dinitrophenyl, 2-aminobenzoyl/2,4-dinitrophenyl and 2-aminobenzoyl/3-nitrotyrosine.

As will be readily understood by a person skilled in the art, a radioactive label can be incorporated within the probe of formula I at any suitable position. For example, a ^{3}H, or ^{14}C isotope can replace any hydrogen or carbon present in the molecule. Similarly, a ^{125}I isotope can be substituted on any aromatic ring.

In principle, these tracer methodologies can easily be adapted for the purpose of high-volume screening. Scintillation proximity assay (SPA) methods for radioactive detection have been developed which do not require a separation step and are easily adapted for robotics and microtiter plate format.

Preferably, the detectable label is a fluorescent label or a chemiluminescent label. More preferably, the label is a fluorescent label. Most preferably, the detectable label is a fluorescein.

Non-radioactive detection methods have become increasingly widespread in screening assay because of the costs associated with radiolabeled reagents and their disposal. Fluorescence spectroscopy is one of the most prevalent non-

radioactive detection methods. One type of assay in which fluorescence may be used is fluorescence polarization. Polarization is independent of total fluorescence intensity; therefore, this technique may not be as prone to interference as fluorescence amplitude measurements. As disclosed herein, the new type of assay developed uses a fluorescein-labeled inhibitor, though other fluorescent labels or non-fluorescent techniques can also be applied.

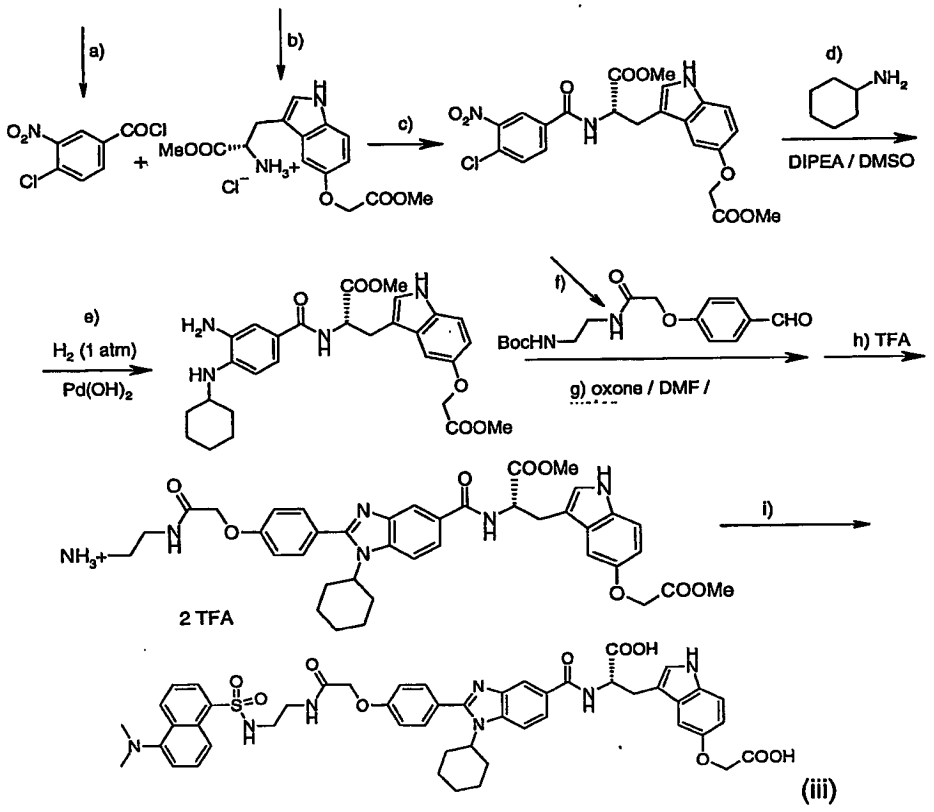
Preferably, the polymerase used in the assay may comprise an affinity tag by which the polymerase can be attached to a solid support, and the probe may be labeled so as to provide a detectable signal. An affinity tag incorporated into the probe maybe a biotin that is used to indirectly measure the association of this biotinylated probe to the NS5B polymerase through the secondary use of an avidin-coupled detection technique.

Preferably, the HCV polymerase used in the present assay is selected from the group consisting of: NS5B; NS5BΔ21; NS5BΔ57 or analogs thereof from a variety of genotypes including HCV-1a or 1b strains having optionally a histidine tag at either the N- or C-terminal. Particularly, as will be understood by a person skilled in the art, this binding assay does not require the polymerase activity of the NS5B to be optimal or functional for such a binding assay to perform according to the invention.

EXAMPLES

Example 1A)

probe (iii): (S)-3-(5-Carboxymethoxy-1H-indol-3-yl)-2-({1-[1-cyclohexyl-2-(4-{[2-(5-dimethylamino-naphthalene-1-sulfonylamino)-ethylcarbamoyl]-methoxy}-phenyl)-1H-benzimidazol-5-yl]-methanoyl}-amino)-propionic acid



a) 4-Chloro-3-nitrobenzoic acid (40.40 g, 0.20 mole) was suspended in DCM (100 mL) containing 3 drops of DMF. Oxalyl chloride (1.5 equivalents, 0.3 mole, 27 mL) was added in small portions and the mixture stirred overnight at room temperature. After refluxing for an additional hour to complete the reaction, volatiles were removed under reduced pressure and the residue was co-evaporated twice with hexane to give the title compound as a light yellow solid.

b) (*S*)-5-Hydroxytryptophan methyl ester hydrochloride (1.55 g, 5 mmol) was dissolved in 80% aqueous MeCN (25 mL) and the solution cooled in ice. Sodium bicarbonate (0.850 g, 10 mmol) was added followed by di-*tert*-butyldicarbonate (1.10 g, 5.1 mmol). The mixture was stirred for 2 h at room temperature, poured into water (200 mL) and extracted with EtOAc (3 X). The combined extracts were washed with water and brine, dried ($MgSO_4$) and concentrated to give a beige solid (1.65 g). The crude product from above (1.50 g, 4.83 mmol) was dissolved in acetone (20 mL) and anhydrous potassium carbonate (1.5 g, 11 mmol) and methyl bromoacetate (0.76 g, 5 mmol) were added. The mixture was reflux for 4 h after which point additional methyl bromoacetate was added to complete the reaction (15 mg portions

until complete by HPLC). The reaction mixture was then cooled and filtered to remove solid. Evaporation of the filtrate gave the desired carbamate as an oil (2.0g). The crude carbamate from above (2.0 g) was deprotected by stirring with 4N HCl – dioxane for 1 h at room temperature. Removal of volatiles in vacuo gave the desired tryptophan ester derivative as a tan-colored solid (1.51 g).

c) The tryptophan derivative from step b) (0.343 g, 1 mmol) was dissolved in 80% aqueous MeCN (10 mL) and sodium bicarbonate (3 equivalents, 0.260 g) was added. The solution was cooled in ice and 4-chloro-3-nitrobenzoyl chloride from step a) (0.220 g, 1 mmol) was added. The mixture was stirred for one hour at room temperature, concentrated under reduced pressure and the residue purified by flash chromatography (1:2 hexane / EtOAc as eluent) to give compound c) as a yellow foam (0.391 g).

d) The 4-chlorobenzamide derivative from above (0.214 g, 0.45 mmol) was dissolved in DMSO (1 mL) and DIEA (0.2 mL) was added followed by cyclohexylamine (3 equivalents, 0.16 mL). The mixture was stirred at 60-65 °C for 4 h and subsequently diluted with water. The orange precipitate that formed was collected, washed with water and dried (0.200 g).

e) The crude material from above (0.200 g, 0.36 mmol) was hydrogenated (1 atm H_2) over 20% $Pd(OH)_2$ on charcoal (60 mg) in MeOH (15 mL). After 2 h, the suspension was filtered to remove the catalyst and concentrated *in vacuo* to give the title compound as a foam (0.16 g).

f) 4-Formylphenoxyacetic acid (0.306 g, 1.70 mmol) was dissolved in DCM (5 mL). DIEA (0.524 g, 4 mmol) and TBTU (0.550 g, 1.70 mmol) were added followed by *tert*-butyl *N*-(2-aminoethyl)carbamate (0.250 g, 1.56 mmol). The mixture was stirred 2 h at room temperature, dissolved in EtOAc and washed sequentially with 5% aqueous K_2CO_3, $KHSO_4$, water and brine. The extract was dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow solid (0.350 g).

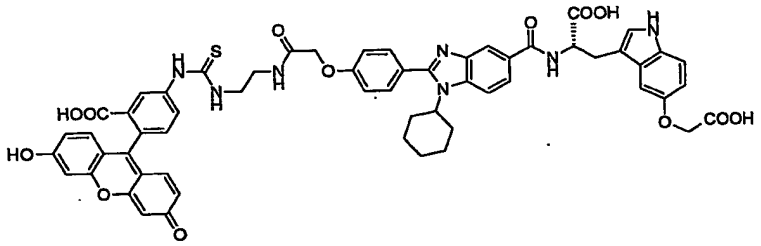
g) The diamine derivative from step e) (0.026 g, 0.05 mmol) and aldehyde from step f) (0.020 g, 0.06 mmol) were dissolved in DMF (0.3 mL) and water (0.03 mL) was added followed by oxone® (0.024 g, 0.04 mmol). The mixture was stirred 1 h at room temperature and then diluted with water. The resulting precipitate was collected by filtration, washed with water and dried to give a beige solid (0.020 g).

h) The crude carbamate from above was stirred with TFA for 30 min at room temperature. Volatiles were removed under reduced pressure and the residue was purified by preparative C18 reversed-phase HPLC to give the bis TFA salt.

i) The amine salt (0.019 g, 0.02 mmol) was dissolved in DMSO (0.3 mL) and DIEA (0.06 mL) was added followed by dansyl chloride (0.065 g, 0.02 mmol). The mixture was stirred for 1 h at room temperature. 5N NaOH (0.12 mL) and water (0.05 mL) were added and the saponification was allowed to proceed for 1 h at room temperature. Following acidification with TFA, the probe (iii) was directly isolated from the reaction mixture by preparative C18 reversed-phase HPLC: MS (ES+) m/z 930 (MH+).

Example 1B)

probe (ii):5-(3-{2-[2-(4-{5-[(S)-1-Carboxy-2-(5-carboxymethoxy-1H-indol-3-yl)-ethylcarbamoyl]-1-cyclohexyl-1H-benzimidazol-2-yl}-phenoxy)-ethanoylamino]-ethyl}-thioureido)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)-benzoic acid

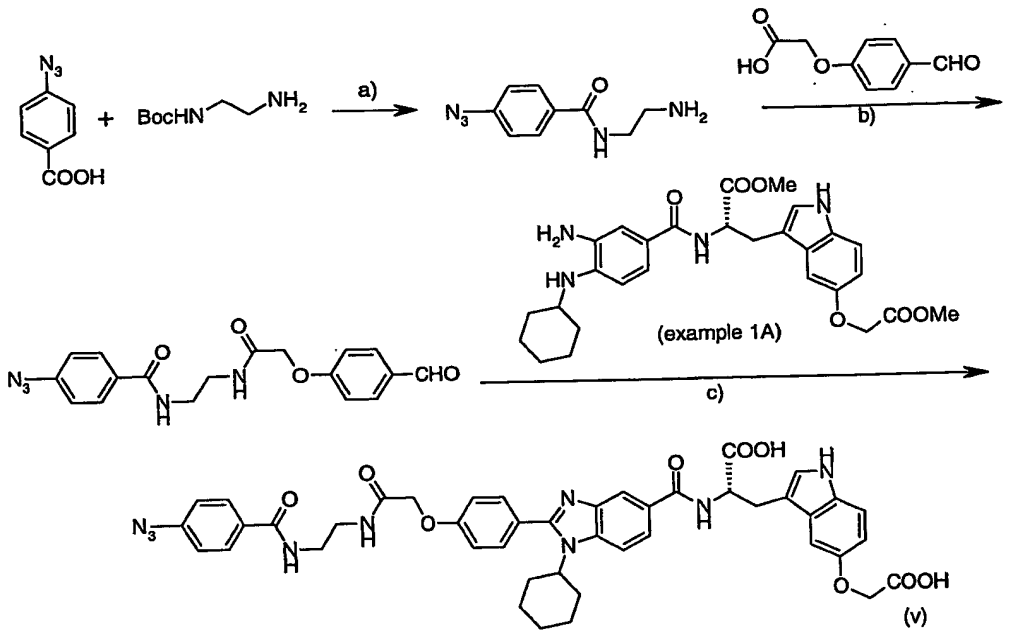


(ii)

The amine salt from step h) of Example **1A** (0.06 mmol) was dissolved in DMSO (0.6 mL) and DIEA (0.3 mL) was added followed by fluorescein isothiocyanate isomer 1 (0.026 g, 0.066 mmol). The mixture was stirred for 1 h at room temperature. 5N NaOH (0.3 mL) and water (0.15 mL) were added and stirring resumed for an additional 30 min. Following acidification with TFA, probe (ii) was isolated directly by preparative C18 reversed-phase HPLC: MS (ES+) m/z 1086 (MH+).

Example 1C)

probe (v): (S)-2-{[1-(2-{4-[(2-{[1-(4-Azido-phenyl)-methanoyl]-amino}-ethylcarbamoyl)-methoxy]-phenyl}-1-cyclohexyl-1H-benzoimidazol-5-yl)-methanoyl]-amino}-3-(5-carboxymethoxy-1H-indol-3-yl)-propionic acid



a) 4-Azidobenzoic acid (0.160 g, 1 mmol) was dissolved in DCM (3 mL). DIEA (0.5 mL, 2.5 mmol) and TBTU (0.337 g, 1.05 mmol) were added followed by *tert*-butyl *N*-(2-aminoethyl)carbamate (0.165 g, 1.03 mmol). The mixture was stirred 2.5 h at room temperature, dissolved in EtOAc and washed sequentially with 5% aqueous K_2CO_3, $KHSO_4$, water and brine. The extract was dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow solid (0.257 g). The crude carbamate (0.257 g, 0.84 mmol) was deprotected by stirring in 4N HCl – dioxane (15 mL) for 2 h at room temperature. Volatiles were removed under reduced pressure to give a pinkish solid.

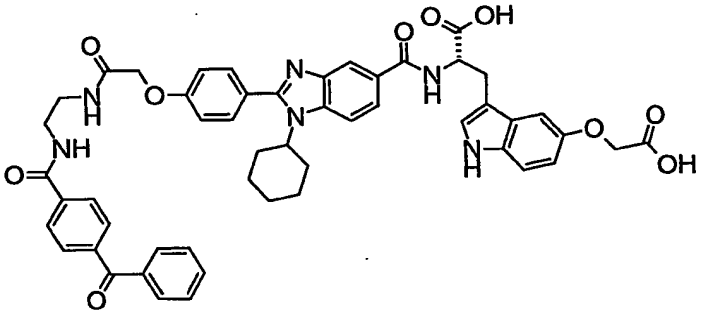
b) 4-Formylphenoxyacetic acid (0.200 g, 1.1 mmol) was dissolved in DCM (3 mL) and DIEA (0.5 mL) was added followed by TBTU (0.350 g, 1,1 mmol) and the amine salt from above (0.240 g, 1 mmol). The mixture was stirred 4 h at room temperature, dissolved in EtOAc and washed sequentially with 5% aqueous K_2CO_3, $KHSO_4$, water and brine. The extract was dried ($MgSO_4$) and concentrated under reduced pressure to give an off-white solid (0.162 g).

c) The benzaldehyde derivative from above (0.044 g, 0.12 mmol) and the diamine derivative from step e) of Example **1A** (0.052 g, 0.1 mmol) were dissolved in DMF (0.6 mL) and water (0.1 mL). Oxone ® (0.050 g, 0.8 mmol) was added and the mixture stirred for 1 h at room temperature. 5N NaOH (0.2 mL) and water (0.1 mL) were added and saponification allowed to proceed for 1 h. Probe (v) was isolated

directly by preparative C18 reversed-phase HPLC (12.5 mg): MS (ES+) m/z 842 (MH+).

Example 1D)

probe (vi): (S)-3-(5-Carboxymethoxy-1H-indol-3-yl)-2-({1-[1-cyclohexyl-2-(4-{[2-({1-[4-(1-phenyl-methanoyl)-phenyl]-methanoyl}-amino)-ethylcarbamoyl]-methoxy}-phenyl)-1H-benzoimidazol-5-yl]-methanoyl}-amino)-propionic acid

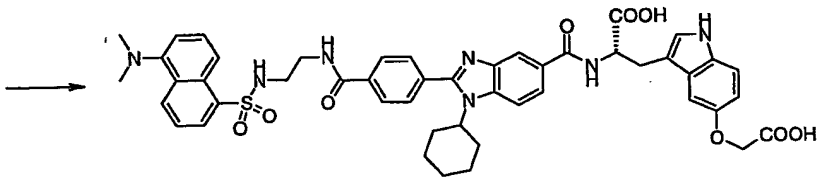


(vi)

Following the procedures described for probe (v) in Example **1C** but using 4-benzoylbenzoic acid instead of 4-azidobenzoic acid, probe (vi) was obtained: MS (ES+) m/z 905 (MH+).

Example 1E)

probe (iv) (S)-3-(5-Carboxymethoxy-1H-indol-3-yl)-2-{[1-(1-cyclohexyl-2-{4-[2-(5-dimethylamino-naphthalene-1-sulfonylamino)-ethylcarbamoyl]-phenyl}-1H-benzimidazol-5-yl)-methanoyl]-amino}-propionic acid



(iv)

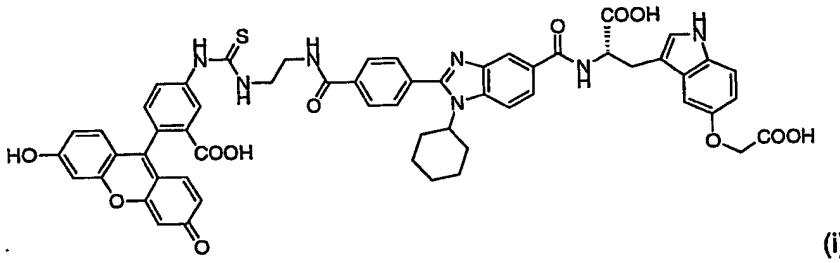
a) Following the procedures described for step f) in Example **1A**, 4-carboxybenzaldehyde was coupled to *tert*-butyl *N*-(2-aminoethyl)carbamate.

b) Following benzimidazole ring formation with the diamine derivative of Example **1A** step e) and the aldehyde from above using oxone® as described in Example **1A** step g), the Boc protecting group was removed and the resulting amine condensed with dansyl chloride as described in Example **1A** step i).

c) Probe (iv) was obtained following saponification of the ester groups under the usual conditions and isolation by preparative C18 reversed-phase HPLC: MS (ES+) m/z 900 (MH+).

Example 1F)

(probe (i):5-[3-(2-{[1-(4-{5-[(S)-1-Carboxy-2-(5-carboxymethoxy-1H-indol-3-yl)-ethylcarbamoyl]-1-cyclohexyl-1H-benzimidazol-2-yl}-phenyl)-methanoyl]-amino}-ethyl)-thioureido]-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)-benzoic acid



(i)

The procedure described for Example **1E)** was used except that fluorescein isothiocyanate isomer 1 was used instead of dansyl chloride. Probe (i) was obtained after purification by preparative C18 reversed-phase HPLC: MS (ES+) m/z 1056 (MH+).

Example 2

Production and purification of HCV NS5B polymerase Δ21-His

The recombinant HCV NS5B polymerase can be produced in soluble form by expression of a variant that lacks the C-terminal 21 amino acids normally found on the mature NS5B (Yamashita *et al.* **1998**, J. Biol. Chem. 273:15479-15486; Ferrari *et al.*, **1999**, J. Virol. 73: 1649-1654). We have expressed this so called NS5BΔ21 with a C-terminal hexa-histidine (termed NS5BΔ21-His; **SEQ ID. NO. 1**) and with an N-terminal hexa-histidine tag (termed His-NS5BΔ21; **SEQ ID NO. 2**) (either proteins being referred to as "his-tag NS5B"). Expression of these genes from pET vectors in E. coli strain JM109 (DE3) is induced with 0.4 mM IPTG for 3 hours at 22 °C. Cells are harvested and lysed in a microfluidizer in lysis buffer (Tris-HCl pH 7.5, 10 % glycerol, 1 mM EDTA, 2 mM 2-mercaptoethanol, 500 mM NaCl, 1 mM PMSF, 1 µg/ml antipain, 1 µg/ml pepstatin A and 1 µg/ml leupeptin). The lysate is clarified by a 30 000 g centrifugation and then supplemented with imidazole to final concentration of 10 mM. The lysate is then loaded onto a metal-chelating resin (Ni-NTA; Qiagen) previously equilibrated with buffer A (Tris-HCl pH 7.5, 10 % glycerol,

500 mM NaCl, 10 mM imidazole), washed extensively and then the protein is eluted with gradient of buffer A containing 500 mM imidazole. Peak fractions containing the his-tag NS5BΔ21 are pooled and diluted with buffer C (20 mM Tris-HCl pH 7.5, 10 % glycerol, 5 mM DTT) to reduce the NaCl concentration to 300 mM and then applied to a DEAE-Sepharose column to remove any nucleic acid. The flow-through from the DEAE-Sepharose column is diluted with buffer C to reduce the NaCl to 200 mM and then applied to a heparin-Sepharose column. The his-tag NS5B is eluted from the heparin-Sepharose in buffer C with a 200 mM to 1 M NaCl gradient. Peak fractions containing the his-tag NS5B are pooled and diluted with buffer C to achieve a final NaCl of 200 mM and loaded onto a Resource S column. Concentrated His-tag NS5B is eluted from the resource S, loaded and size fractionated on a Superdex 200 column in buffer C containing 300 mM NaCl. Peak fractions contain highly pure his-tag NS5B and are stored at –80 °C until use.

Example 3

Fluorescence anisotropy analysis

Titration of the probe with the enzyme was performed as follows:

The fluorescein labeled probe was diluted to the desired concentration in 20 mM Tris-HCl pH 7.5, 1mM EDTA, 5 mM $MgCl_2$, 1 mM DTT and 10% DMSO. The NS5BΔ21-His protein was serially diluted in 25 mM Tris-HCl pH 7.5, 300 mM NaCl, 5 mM DTT, 1 mM EDTA, 30% glycerol and 0.1% IGEPAL. Total volume of the reaction was 500 µL and final assay buffer was 20 mM Tris-HCl pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT, 30 mM NaCl, 3% glycerol, 0.01% IGEPAL and 5% DMSO. Anisotropy measurements were performed on a SLM Aminco 8100 Spectrofluorometer equipped with a 450-W xenon arc lamp and a T-optics configuration. Excitation wavelength was at 493 nm and emission was monitored at 530 nm. In each anisotropy measurement, the parallel and perpendicular intensities of the background buffer solution was subtracted from the measured values of the sample and the anisotropy was calculated. Data were processed on SAS program (SAS Institute Inc., NC, USA) for a non linear regression to obtain the direct binding equilibrium constant and other parameters, and the plot of the regressed fit over the experimental data. An example of a titration curve obtained with probe (i) is shown on Figure 1. K_d values for probes (i) and (ii) with the polymerase were respectively of 15 nM and 6 nM.

Example 4

96-well plate Polarization assay

To obtain K_d values of different compounds competing with these probes (test compounds), this assay was transformed to a more amenable format and a binding assay was made suitable for a 96-well microplate reader. The probe was diluted in order to obtain the desired final concentration (from 4 to 25 nM, depending on its K_d towards the enzyme and on the conditions of the assay). The tested compounds were serially diluted by a factor of 2 or 3-fold in 20 mM Tris-HCL pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT and 15% DMSO. The NS5BΔ21-His concentration in the assay was calculated to obtain 70% of binding of the probe; these conditions allowed for the displacement of the probe by test compounds. The assay reactions finally contained 50 µL of the serial dilutions of the tested compounds that were transferred in 96-well black plates (Packard); a complete row was however free of compound to obtain a positive control value and verify real percent of bound probe in the experiment. 50 µL of the probe were then added to each well, except in one column for blank subtraction. Lastly, 150 µL of enzyme were added to all wells, except in one row, which was used to determine the 0% and 100% bound values. In this row of 8 wells, enzyme buffer was added to the first 4 wells (to determine the anisotropy value of the free probe or r_f) and a 10-fold excess of the concentration of the enzyme used in the assay was added to the other 4 wells (to determine the anisotropy value when 100% of the probe is bound i.e. the r_b value). These values were required to calculate the K_d values. The final buffer conditions of the assay were identical to the ones used for K_d determination of the probes, i.e. 20 mM Tris-HCl pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT, 30 mM NaCl, 3% glycerol, 0.01% IGEPAL and 5% DMSO. The reactions were incubated for 90 minutes at room temperature in the dark. Readings of polarization were then performed on a POLARstar Galaxy, equipped with a high-energy xenon flash lamp, using an excitation filter of 485 nm and an emission filter of 520 nm. Polarization values can be converted easily to anisotropy values with the following calculation (Owicki *et al.*, **2000**, J. Biomol. Screen. 5:297-306):

$$a = 2 \times P / (3 - P) \text{ where}$$

a: anisotropy value

P: Polarization value

Anisotropy values can then be used to obtain two types of results

fitted to SAS nonlinear regression analysis to obtain apparent K_d values, using for the calculations as positive control the anisotropy value at ~ 70% binding, and as negative control the anisotropy value of the free probe (r_f);

fitted to the Anisotropy equation:

$$a = \frac{(-K_d - I + E_o) + \sqrt{((K_d + I - E_o)^2 + 4 * K_d * E_o)}}{2[\frac{(a * Q * r_b + K_p * r_f)}{(K_p + a * Q)}]}$$

where a: anisotropy

K_d: dissociation constant for the inhibitor

I: Concentration of compound (or inhibitor) tested

E_o: NS5B concentration (E_o has to be >> [probe])

$Q = Q_b/Q_f$ = total fluorescence for probe 100% bound/ total fluorescence for free probe

r_b: anisotropy value when the probe is 100% bound

r_f: anisotropy value when the probe is free

K_p: dissociation constant for the probe

This high throughput assay was evaluated and validated by the determination of the statistical parameter Z' (J.-H. Zhang *et al.*, **1999**, J. of Biomol. Screening, 4:67-73). Results of this experiment are illustrated on Figure 2. The anisotropy values for a series of positive and negative controls were very similar, resulting in very low standard deviations; 0.2186±0.0036 A units for the positive controls and 0.0738±0.0037 A units for the negative controls. The Z' value obtained for the assay was of 0.85, implying that we have excellent conditions to detect compounds that would compete with the probe.

Example 5

Inhibitor testing

We have identified potent compounds that can effectively displace the probe in this binding assay. Figures 3 and 4 show examples of some of them, with K_d values ranging from 31 nM to 1 µM. The anisotropy equation was defined in the Grafit Software (Erithacus Software Ltd., UK) and plotted such that inhibitor concentration was the X-variable and anisotropy was the Y variable; parameters calculated by the

software were the inhibitor Kd and Qb/Qf ratio. Supplied constants were the K_p, E_0, r_b and r_f.

Example 6

Modified conditions for the Polarization assay

The usefulness of this polarization assay is evident when binding of compounds has to be studied under different conditions. For example, binding constants of the probes have been determined at different concentrations of salts and pH. Figures 5 to 8 show the binding curves of probe (i) in final NaCl concentrations ranging from 30 mM to 200 mM. All other reagents in the assay were as described in the standard protocol (Example 3). As shown on these Figures, K_d values gradually increase with salt concentration from Kd=15 nM (at 30 mM NaCl) to Kd=122 nM (at 200 mM NaCl). Studies at pH 6.5 were also performed to determine the K_d of the probe (i) at lower pH. For these assays, 20 mM Phosphate buffer pH 6.5 was used in place of Tris; all other reagents of the assay were as described in the 96-well Polarization assay (Example 4). An example of these types of experiments is shown in Figure 9. The K_d value obtained at pH 6.5 with probe (i) was of 33 nM. Having established these K_d values under different experimental conditions, it is then trivial to determine what concentrations of probe and enzyme should be used to obtain 70% of binding of the probe with the equilibrium equation. Once these values are obtained, compounds of interest can easily be studied under the new conditions to determine their K_d values.

Example 7

Fluorescence Polarization assay with a modified enzyme

The Fluorescence polarization assay was also used with other constructs of our HCV polymerase enzyme. In addition to the C-terminally tagged NS5BΔ21-His polymerase, the NS5B enzyme with the His-tag at the N-terminal position was also used in the fluorescence polarization assay. Determination of the K_d for the probe (i) with this enzyme was performed, using the same conditions described in the standard 96-well format assay. Figure 10 shows that the K_d obtained with probe (i) was similar, i.e. 18 nM. A comparison was made between the IC_{50} and the K_d for three compounds, using these two different constructs of the enzymes (NS5BΔ21-His and His-NS5BΔ21).

IC_{50}'s are determined using the Scintillation Proximity Assay (SPA) according to the following assay:

The substrates are: (i) a 12 nucleotide RNA oligo-uridylate (or oligo-uridine-monophosphate) (oligo-U) primer modified with biotin at the free 5'C position; (ii) a complementary poly-adenylate (or adenosine monophospahte) (polyA) template of heterogeneous length (1000-10000 nucleotides); and (iii) UTP-[5,6 ^{3}H]. Polymerase activity is measured as the incorporation of UMP-[5,6 ^{3}H] into the chain elongated from the oligo-U primer. The ^{3}H-labelled reaction product is captured by SPA-beads coated with streptavidin and quantified on the TopCount (Packard). Inhibitors are tested at various concentrations in a reaction containing: 1 to 5 nM of the his-tagged NS5B, 1 µg/ml of biotinylated oligo U primer, 10 µg/ml of polyA template, 20 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 25 mM KCl, 1 mM EDTA, 1 mM DTT, 0.33 % n-dodecyl maltoside, 5% DMSO, 0.0083 µCi/µl [0.25 µM] UTP-[5,6-^{3}H], 0.75 µM UTP, 1.67 U/ µl RNAsin™. The reaction was incubated at room temperature for 1.5 hours. STOP solution (20 µl; 0.5 M EDTA, 150 ng/ µl tRNA) was added, followed by 30 µl streptavidin coated PVT beads (8mg/ml in 20 mM Tris-HCl, pH 7.5, 25 mM KCl, 0.025% NaN_3). The plate was then shaken for 30 minutes. A solution of CsCl was added (70 µl, 5 M), to bring the CsCl concentration to 1.95 M. The mixture was then allowed to stand for 1 hour. The beads were then counted on a Hewlett Packard TopCount™ instrument. Based on the results at ten different concentrations of test compound, standard concentration-% inhibition curves were plotted and analysed to determine IC_{50}'s for the compounds.

Results of this experiment are illustrated in Table I. The K_d values were similar with both enzymes for the three compounds tested, whereas the IC_{50} values obtained with the two enzymes show significant differences and reflect the differences in substrate affinity.

Example 8

Specificity of the Fluorescence Polarization assay

The utility of the Fluorescence polarization assay was examined with another distantly related viral polymerase and with a closely related genotype (1a) HCV polymerase.

The GBV-B polymerase enzyme (termed GBV-BΔ23-His; **SEQ ID NO. 3**) (Simons,

J.N. *et al.*, **1995**, Proc. Natl. Acad. Sci. USA 92, 3401-3405; Bukh, J. *et al.*, **1999**, Virology 262, 470-478) was produced and purified as described in Example 2 with the following modifications:

Expression of the gene from pET vectors in E. coli strain JM109 (DE3) was induced with 0.5 mM IPTG for 3 hours at 22 °C. Cells were harvested and lysed in a microfluidizer in buffer A (Tris-HCl pH 7.5, 10 % glycerol, 1 mM EDTA, 2 mM 2-mercaptoethanol, 500 mM NaCl, 1 mM PMSF, 1 ug/ml antipain, 1 ug/ml pepstatin A , 1 ug/ml leupeptin and 0.5% dodecyl-β-D-maltoside). The lysate was clarified by a 30 000 g centrifugation and then supplemented with imidazole to a final concentration of 10 mM. The lysate was then loaded onto a metal-chelating resin (Ni-NTA; Qiagen) previously equilibrated with buffer A containing 10 mM imidazole, washed extensively and then the protein was eluted with a gradient of buffer A containing 500 mM imidazole. Peak fractions containing the his-tag GBV-BΔ23 were pooled and diluted with buffer C (20 mM Tris-HCl pH 7.5, 10 % glycerol, 5 mM DTT, 0.01% dodecyl-β-D-maltoside) to reduce the NaCl concentration to 300 mM and then applied to a DEAE-Speharose column to remove any nucleic acid. The flow-through from the DEAE-Speharose column was diluted with buffer C to reduce the NaCl to 200 mM and then applied to a heparin-Sepharose column. The his-tag GBV-B was eluted from the heparin-Sepharose in buffer C with a 200 mM to 1 M NaCl gradient. Peak fractions containing the pure his-tag GBV-B were then pooled and stored at –80 °C until use.

The HCV genotype 1a NS5B polymerase [termed His-NS5BΔ21(H77c,1a); **SEQ ID NO. 4**] (Yanagi, M. *et al.*, **1997**, Proc. Natl. Acad. Sci. USA 94, 8738-8743) was produced and purified as described in Example 2 with the following modifications:

Expression of the gene from pET vectors in E. coli strain JM109 (DE3) was induced with 0.4 mM IPTG for 3 hours at 22 °C. Cells were harvested and lysed in a microfluidizer in buffer A (Tris-HCl pH 8.0, 10 % glycerol, 1 mM EDTA, 2 mM 2-mercaptoethanol, 500 mM NaCl, 1 mM PMSF, 1 ug/ml antipain, 1 ug/ml pepstatin A, 1 ug/ml leupeptin, 1% dodecyl-β-D-maltoside, 1% Triton X-100 and 0.1% CHAPS). The lysate was clarified by a 30 000 g centrifugation and then supplemented with imidazole to a final concentration of 10 mM. The lysate was then loaded onto a metal-chelating resin (Ni-NTA; Qiagen) previously equilibrated with buffer A containing 10 mM imidazole, 0.1% NP-40, without CHAPS, and with lower

concentrations of the other detergents (0.2% dodecyl-β-D-maltoside, 0.05% Triton X-100); after extensive washing, the protein was eluted with a gradient of buffer A containing 500 mM imidazole. Peak fractions containing the his-tag NS5BΔ21(H77c,1a) were pooled and diluted with buffer C (20 mM Tris-HCl pH 8.0, 10 % glycerol, 5 mM DTT, 0.2% dodecyl-β-D-maltoside) to reduce the NaCl concentration to 300 mM and then applied to a DEAE-Sepharose column to remove any nucleic acid. The flow-through from the DEAE-Sepharose column was diluted with buffer C to reduce the NaCl to 200 mM and then applied to a heparin-Sepharose column. The his-tag NS5BΔ21(H77c,1a) was eluted from the heparin-Sepharose in buffer C with a 200 mM to 1 M NaCl gradient. Peak fractions containing the polymerase were then pooled and diluted with buffer C to achieve a final NaCl of 200 mM and loaded onto a Resource S column. Peak fractions containing the his-tag NS5B(H77c,1a) were pooled, loaded and size fractionated on a Superose 12 column in buffer C containing 600 mM NaCl. Peak fractions contain highly pure his-tag NS5B were pooled and stored at –80 °C until use.

The GBV-B and the HCV 1a polymerases were used to titrate probe ii, using the protocol described in Example 3. Figures 11 and 12 show the titration curves observed with the GBV-B polymerase and the NS5B(H77c,1a) polymerase, respectively. The K_d value of probe ii for the GBV-B enzyme was 1.8 uM (estimated value with an incomplete curve and an r_b value of 0.21), illustrating the weak binding of the probe to this distantly related polymerase. In contrast, the K_d for the HCV 1a polymerase was 18 nM, revealing that the 1a genotype enzyme binds probe ii with the same affinity as the HCV 1b genotype polymerase.

K_d values for a series of compounds were determined with these two HCV (genotypes 1a and 1b) polymerases, using the assay format described in Example 4.

Results of this experiment are illustrated in Table 2. These results show that the K_d values for this series of inhibitors are in the same range with the two genotypically related HCV enzymes.

TABLE 1

Comparison of compound K_d and IC_{50} values with two different HCV NS5B polymerases

Cpd	K_d value (nM)		IC_{50} value (nM)	
	NS5BΔ21-His	His-NS5BΔ21	NS5BΔ21-His	His-NS5BΔ21
X	44	41	867	66
Y	22	31	348	68
Z	92	88	735	34

TABLE 2

Comparison of compound K_d values with NS5B polymerases from two HCV genotypes

Cpd	K_d values (nM)	
	His-NS5BΔ21(1b)	His-NS5BΔ21(H77c,1a)
A	2.7	1.8
B	12	8.0
C	5.3	7.2
D	3.5	7.1
E	2.4	2.7

DISCUSSION

The HCV NS5B polymerase is a prime target in the search for inhibitors of HCV replication. The HCV NS5B enzymatic activity has been studied *in vitro* with a variety of RNA substrates (Behrens et al., **1996**; and many references thereafter). Different preparations of the HCV polymerase exhibit varying efficiencies of product formation with a variety of RNA substrates. Estimations are that only a small fraction (i.e. < 1%) of the common preparations of purified recombinant HCV NS5B polymerase interact

with RNA substrate to reconstitute processive RNA product synthesis (Carroll SS, *et al.*, **2000**. Biochemistry, 39:8243-8249). Moreover, the activity of purified recombinant NS5B polymerase varies significantly with specific RNA substrates; a characteristic that presumably reflects the capability of the NS5B of forming productive replication-competent complexes with these substrates (Zhong W, et al., **2000,** J Virol, 74, 9134-9143).

In an effort to overcome the limitations of HCV polymerase assays that use sub-optimal and poorly characterized RNA substrates, the Applicants have developed an assay for specific inhibitors of the HCV polymerase that is independent of the presence of RNA. The assay is based upon the use of a characterized inhibitor specific for the HCV polymerase. In the examples presented above, the inhibitor was labeled with a fluorescein moiety and the interaction of this probe with the NS5B was measured and quantified by fluorescence polarization. However, the interaction can also be measured by the use of a radiolabel, or other common labels placed on the inhibitor and applying common techniques for assessing the association of the labeled probe with an appropriately tagged target HCV polymerase. Binding equilibrium with the fluorescein labeled probe is clearly evident in Example 3, as the fraction of bound probe increased with the amount of HCV polymerase. An HCV polymerase assay with components at equilibrium is an advantage over previous assays with RNA substrates, as the active HCV polymerase that stably associates with RNA substrates in processive complexes does not readily dissociate (Carroll SS, *et al.*, **2000** Biochemistry, 39:8243-8249; Zhong W, *et al.*, **2000** J Virol, 74, 9134-9143; Tomei L, *et al.* **2000** J Gen. Virol. 81, 759-767.). Though these labeled probes readily dissociate from the HCV polymerase, they do so with low nM dissociation constants and provide the required sensitivity (in the low nM range) to detect potent and specific inhibitors. The assay format is adaptable to screening in 96-well (or higher density) plate format as demonstrated in Example 4. A particular advantage of this high throughput screening format is the extremely stable signal and minimal well-to-well variation that the assay provides, particularly in a convenient non-radioactive format. Specific inhibitors of the HCV polymerase were identified and potencies easily determined with this assay (Figures 3 and 4).

The direct binding assay described herein overcomes other limitations of the enzymatic HCV polymerase assay. The *in vitro* RNA polymerase activity of NS5B is

extremely sensitive to ionic strength, and KCl or NaCl concentrations exceeding 100 mM inhibit the reaction (Lohmann V, *et al.*, **1998** Virology 249, 108-118; Luo G, *et al.*, **2000**, J Virol., 74, 851-63.) Hence the ability to determine the potency of inhibitors at various salt concentrations is restricted by this limitation of standard enzymatic reactions. The direct binding assay of this invention is amenable to adjustments in salt concentration or pH levels as demonstrated in Example 6. The potencies and interaction of specific inhibitors with the NS5B target can easily be determined under conditions not suitable for enzymatic RNA polymerization studies (such as the absence of divalent cation).

Established HCV polymerase enzymatic assays provide IC_{50} values as representative measurements of inhibitor potencies. For inhibitors that are competitive with either RNA or NTP, the IC_{50} value is proportional to the concentration of substrates in the assay and will vary depending on the concentration of these components. The assay described herein permits a direct measurement of inhibitor potencies (reflected by Kd values), under defined conditions, irrespective of the substrate concentration. In enzymatic reactions that use either the N-terminal tag His-NS5BΔ21 or the C-terminal tag NS5BΔ21-His, significantly disparate IC_{50} values are obtained for identical compounds assayed under identical conditions. The His-NS5BΔ21 and NS5BΔ21-His polymerases have different affinities for the primer/template RNA substrate thereby resulting in the disparate IC_{50} for the identical compounds (Example 7, Table 1). A major advantage that is exemplified by the direct binding assay described in this invention is that these differences are reconciled by the relatively similar K_d values that the individual inhibitors display with the two different HCV polymerases.

The direct binding assay described herein has also been shown to be specific for HCV polymerase enzymes. Example 8, in which a K_d at least 100-fold higher for the probe ii was obtained with the GBV-B polymerase, illustrates the weak binding of the probe to this polymerase and the specificity of binding to the HCV polymerases. Moreover, Example 8 also demonstrates that the polymerases from two distinct and clinically relevant HCV genotypes bind the probe with similar affinities.

The direct inhibitor-binding assay of this invention alleviates many restrictions of conventional HCV polymerase enzymatic assays described to date. The Applicants have exemplified how the use of a characterized inhibitor as a competitive probe provides a number of improvements and advancements in the search for specific inhibitors of the NS5B polymerase. This assay may accelerate the identification and characterization of candidate therapeutics for the treatment of HCV related diseases.

CLAIMS

1. A method for identifying compounds binding to HCV polymerase comprising the steps of:

a) contacting said HCV polymerase or an analog thereof with a probe being capable of binding to an HCV polymerase or an analog thereof, said probe being displaceable by an inhibitor thereof, so as to form a complex comprising said probe bound to said polymerase;

b) measuring a signal emitted from said probe in said complex to establish a base line level;

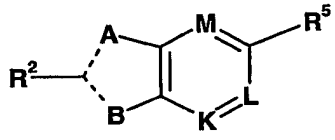
c) incubating the product of step a) with a test compound; and

d) measuring the signal from said complex; and

e) comparing the signal from step d) with the signal from step b);

whereby a modulation in said signal is an indication that said test compound binds to said polymerase.

2. The method according to claim 1, wherein said probe is selected from: an isomer, enantiomer, diastereoisomer, or tautomer of a probe represented by formula I:



(I)

wherein **A** is O, S, N, NR^1, or CR^1, wherein R^1 is selected from the group consisting of: H, (C_{1-6})alkyl optionally substituted with:

-halogen, OR^{11}, SR^{11} or $N(R^{12})_2$, wherein R^{11} and each R^{12} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**, said aryl or **Het** optionally substituted with R^{10}; or

both R^{12} are covalently bonded together and to the nitrogen to which they are both attached to form a 5, 6 or 7-membered saturated heterocycle;

----- represents either a single or a double bond;

R^2 is selected from: H, halogen, R^{21}, OR^{21}, SR^{21}, $COOR^{21}$, $SO_2N(R^{22})_2$, $N(R^{22})_2$, $CON(R^{22})_2$, $NR^{22}C(O)R^{22}$ or $NR^{22}C(O)NR^{22}$ wherein R^{21} and each R^{22} is

independently H, (C_{1-6})alkyl, haloalkyl, (C_{2-6})alkenyl, (C_{3-7})cycloalkyl, (C_{2-6})alkynyl, (C_{5-7})cycloalkenyl, 6 or 10-membered aryl or **Het**, said R^{21} and R^{22} being optionally substituted with R^{20};

or both R^{22} are bonded together to form a 5, 6 or 7-membered saturated heterocycle with the nitrogen to which they are attached;

B is NR^3 or CR^3, wherein R^3 is selected from (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl, (C_{6-10})bicycloalkyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**, said alkyl, cycloalkyl, bicycloalkyl, aryl, **Het**, alkyl-aryl and alkyl-**Het** being optionally substituted with from 1 to 4 substituents selected from: halogen, or

a) (C_{1-6})alkyl optionally substituted with:

- OR^{31} or SR^{31} wherein R^{31} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; or
- $N(R^{32})_2$ wherein each R^{32} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; or both R^{32} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

b) OR^{33} wherein R^{33} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**;

c) SR^{34} wherein R^{34} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; and

d) $N(R^{35})_2$ wherein each R^{35} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**; or both R^{35} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

with the proviso that when **A** is not N, then one of **A** or **B** is either CR^1 or CR^3;

K is N or CR^4, wherein R^4 is H, halogen, (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or R^4 is OR^{41} or SR^{41}, COR^{41} or $NR^{41}COR^{41}$ wherein each R^{41} is independently H, (C_{1-6})alkyl), (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl;

or R^4 is $NR^{42}R^{43}$ wherein R^{42} and R^{43} are each independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, or both R^{42} and R^{43} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

L is N or CR^5, wherein R^5 has the same definition as R^4 defined above;

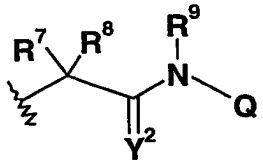
M is N or CR^7, wherein R^7 has the same definition as R^4 defined above;

R^5 is $C(Y^1)Z$ wherein Y^1 is O or S;

Z is $N(R^{6a})R^6$ or OR^6, wherein R^{6a} is H or (C_{1-6})alkyl or $NR^{61}R^{62}$ wherein R^{61} and R^{62} are each independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, or both R^{61} and R^{62} are covalently bonded together and to the nitrogen to which they are both attached to form a 5, 6 or 7-membered saturated heterocycle; or R^{62} is $COOR^{63}$ wherein R^{63} is (C_{1-6})alkyl, (C_{3-6})cycloalkyl, said alkyl or cycloalkyl being optionally substituted with 6- or 10-membered aryl or **Het**; or R^{62} is COR^{64} wherein R^{64} is $C_{1-6})$alkyl, (C_{3-6})cycloalkyl -6-or 10-membered aryl or **Het**; and

R^6 is selected from the group consisting of: H, (C_{1-6})alkyl, (C_{3-6})cycloalkyl, (C_{2-6})alkenyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het**, wherein said alkyl, cycloalkyl, alkenyl, aryl, **Het**, alkyl-aryl, or alkyl-**Het**, are all optionally substituted with R^{60};

or R^6 is



wherein R^7 and R^8 are each independently H, (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het**, wherein said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het** are optionally substituted with R^{70};

or

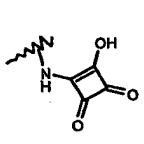
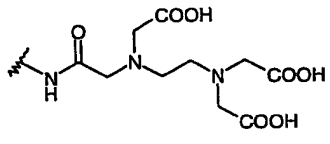
R^7 and R^8 are covalently bonded together to form second (C_{3-7})cycloalkyl or a 4, 5- or

6-membered heterocycle having from 1 to 4 heteroatom selected from O, N, and S; or when **Z** is $N(R^{6a})R^6$, either of R^7 or R^8 is covalently bonded to R^{6a} to form a nitrogen-containing 5-or 6-membered heterocycle;

Y^2 is O or S;

R^9 is H, (C_{1-6} alkyl), (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl or (C_{1-6})alkyl-**Het**, all of which optionally substituted with R^{90}; or
R^9 is covalently bonded to either of R^7 or R^8 to form a 5- or 6-membered heterocycle;

Q is a 6- or 10-membered aryl, **Het**, (C_{1-6}) alkyl-CONH-aryl or (C_{1-6}) alkyl-CONH-**Het**, all of which being optionally substituted with:



, or R^{100};

or a salt or a derivative thereof;

wherein **Het** is defined as 5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N, and S, or a 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N and S; and

R^{10}, R^{20}, R^{60}, R^{70}, R^{90} and R^{100} is each defined as:

- 1 to 4 substituents selected from: halogen, OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl,

Het, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H,

(C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150}; and

wherein R^{150} is defined as:

- 1 to 3 substituents selected from: halogen, OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl,

aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted

with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160}; and

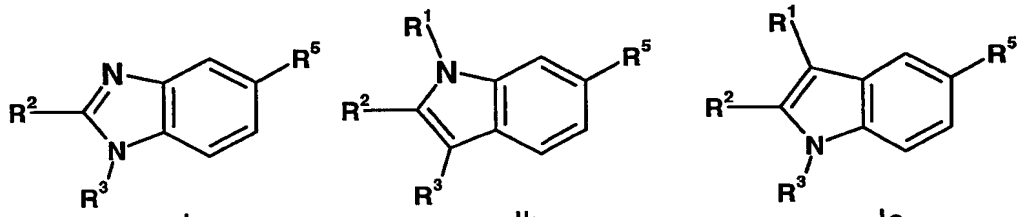
k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SR^{161}, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are

covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

wherein said probe comprises a detectable label attached to any suitable position, whereby said probe binds to an HCV polymerase or an analog thereof and is capable of being displaced by an inhibitor thereof;

3. The method according to claim 2, wherein said probe has the following formula:



Ia Ib or Ic

wherein

R^1 is selected from the group consisting of: H or (C_{1-6})alkyl;

R^2 is $CON(R^{22})_2$, wherein each R^{22} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{5-7})cycloalkenyl, 6 or 10-membered aryl or **Het**, or both R^{22} are bonded together to form a 5, 6 or 7-membered saturated heterocycle with the nitrogen to which they are attached;

or R^2 is selected from: H, halogen, (C_{1-6})alkyl, haloalkyl, (C_{2-6})alkenyl, (C_{5-7})cycloalkenyl, 6 or 10-membered aryl or **Het**; wherein each of said alkyl, haloalkyl, (C_{2-6})alkenyl, (C_{5-7})cycloalkenyl, aryl or **Het** is optionally substituted with R^{20}, wherein R^{20} is defined as:

- 1 to 4 substituents selected from: halogen, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**,

(C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, (C1-6alkyl)aryl or (C1-6alkyl)**Het**, all of which being optionally substituted with R^{150};

wherein R^{150} is preferably:

- 1 to 3 substituents selected from: halogen, NO_2, cyano or azido; or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl) or (C_{3-7})cycloalkyl, said alkyl or cycloalkyl optionally substituted with R^{160};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het** and

heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, or (C_{3-7})cycloalkyl, and R^{112} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl said (C_{1-6})alkyl and (C_{3-7})cycloalkyl being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

said alkyl, cycloalkyl, and heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl and cycloalkyl being optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl) or (C_{3-7})cycloalkyl, or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl and cycloalkyl being optionally substituted with R^{160};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said (C_{1-6})alkyl and (C_{3-7})cycloalkyl being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6

or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

R^3 is selected from (C_{3-7})cycloalkyl, (C_{6-10})bicycloalkyl, 6- or 10-membered aryl, or **Het**;

R^5 is -C(O)-**Z**, wherein

Z is OR^6 wherein R^6 is C_{1-6}alkyl substituted with:

- 1 to 4 substituents selected from: OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is (C_{1-6}alkyl) substituted with R^{150}, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

d) SR^{108}, SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-}

$_6$alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is CN, (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150}; or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-}

$_{7}$)cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** said alkyl, cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** said (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** all of which being optionally substituted with R^{150}; wherein R^{150} is:

- 1 to 3 substituents selected from: halogen, NO_2, cyano, azido or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115}

is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-}$

$_6$alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or **Z** is $N(R^{6a})R^6$, wherein R^{6a} is H or (C_{1-6}alkyl) and

R^6 is (C_{1-6})alkyl optionally substituted with:

- 1 to 4 substituents selected from: OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl substituted with R^{150a}, haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150}, wherein R^{150a} is the same as R^{150} but is not halogen, OR^{150b}, $COOR^{150b}$, $N(R^{150b})_2$, wherein R^{150b} is H or C_{1-6}alkyl;

b) OR^{104} wherein R^{104} is (C_{1-6}alkyl) substituted with R^{150}, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl,

Het, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** or

R^{111} is H and R^{112} is SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;
said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};
or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are

covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** said (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het,** (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het,** (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150}; and

wherein R^{150} is selected from:

- 1 to 3 substituents selected from: halogen, NO_2, cyano, azido or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or

heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, and R^{112} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl optionally substituted with R^{160};

or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

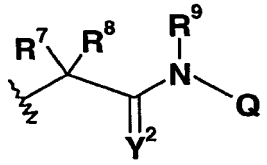
j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**,

said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both R^{162} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^6 is



wherein, preferably, R^7 and R^8 are each independently H, (C_{1-6})alkyl, haloalkyl, (C_{3-7})cycloalkyl, 6- or 10-membered aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het**, wherein said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6})alkyl-aryl, (C_{1-6})alkyl-**Het** are optionally substituted with R^{70}; or

R^7 and R^8 are covalently bonded together to form second (C_{3-7})cycloalkyl or a 4, 5- or 6-membered heterocycle having from 1 to 3 heteroatom selected from O, N, and S; or when **Z** is $N(R^{6a})R^6$, either of R^7 or R^8 is covalently bonded to R^{6a} to form a nitrogen-containing 5-or 6-membered heterocycle;

wherein R^{70} is selected from:

- 1 to 4 substituents selected from: halogen, NO_2, cyano, azido; or

- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

d) $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-}

$_6$alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

and R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, $(C_{1-6}$alkyl)aryl or (C1-6alkyl)**Het**, all of which being optionally substituted with R^{150};

wherein R^{150} is selected from:

- 1 to 3 substituents selected from: halogen, NO_2, cyano, azido; or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-6})alkynyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, $(C_{1-6}$alkyl) or (C_{3-7})cycloalkyl, said alkyl and cycloalkyl being optionally substituted with R^{160};

d) $SO_2N(R^{108})_2$ wherein R^{108} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl or cycloalkyl being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, and R^{112} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het**, $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said (C_{1-6})alkyl or (C_{3-7})cycloalkyl being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl; or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl or heterocycle being optionally substituted with R^{160};

h) $NR^{121}COCOR^{122}$ wherein R^{121} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said alkyl or cycloalkyl being optionally substituted with R^{160}; or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl) or (C_{3-7})cycloalkyl, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

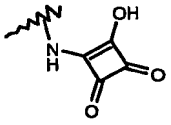
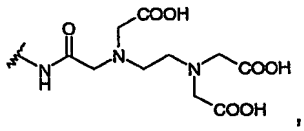
j) tetrazole, $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, said (C_{1-6})alkyl and (C_{3-7})cycloalkyl being optionally substituted with R^{160}; and

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl or (C_{3-7})cycloalkyl, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl and heterocycle being optionally substituted with R^{160};

wherein R^{160} is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, OR^{161}, $N(R^{162})_2$ or $CON(R^{162})_2$, wherein R^{161} and each R^{162} is independently H or (C_{1-6})alkyl;

R^9 is H; or R^9 is covalently bonded to either of R^7 or R^8 to form a 5- or 6-membered heterocycle; and

Q is a 6- or 10-membered aryl, **Het**, all of which being optionally substituted with:

, , or R^{100};

wherein R^{100} is:

- 1 to 4 substituents selected from: halogen, NO_2, cyano or azido; or
- 1 to 4 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** , $COOR^{115}$ or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het,** or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle; said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150}; or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{124} is OH or $O(C_{1-6}$alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, all of which being optionally substituted with R^{150};

wherein R^{150} is selected from:

- 1 to 3 substituents selected from: halogen, NO_2, cyano or azido; or
- 1 to 3 substituents selected from:

a) (C_{1-6}) alkyl or haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl

optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-6})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{160};

b) OR^{104} wherein R^{104} is H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** or heterocycle being optionally substituted with R^{160};

e) $NR^{111}R^{112}$ wherein R^{111} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** or SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or heterocycle being optionally substituted with R^{160};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{160};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^{119} and R^{120} are covalently bonded together and to the nitrogen to

which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with $\mathbf{R^{160}}$;

h) $NR^{121}COCOR^{122}$ wherein $\mathbf{R^{121}}$ is H, (C_{1-6})alkyl optionally substituted with $\mathbf{R^{160}}$;

or $\mathbf{R^{122}}$ is OR^{123} or $N(R^{124})_2$ wherein $\mathbf{R^{123}}$ and each $\mathbf{R^{124}}$ is independently H, $(C_{1-6}$alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or $\mathbf{R^{124}}$ is OH or $O(C_{1-6}$alkyl) or both $\mathbf{R^{124}}$ are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with $\mathbf{R^{160}}$;

j) tetrazole, $COOR^{128}$ wherein $\mathbf{R^{128}}$ is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl and $(C_{1-6}$alkyl)**Het** being optionally substituted with $\mathbf{R^{160}}$; and

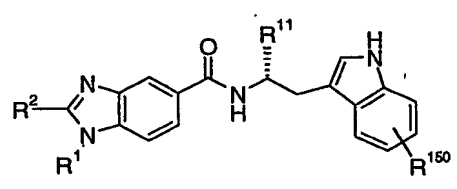
k) $CONR^{129}R^{130}$ wherein $\mathbf{R^{129}}$ and $\mathbf{R^{130}}$ are independently H, $(C_{1-6}$alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both $\mathbf{R^{129}}$ and $\mathbf{R^{130}}$ are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** and heterocycle being optionally substituted with $\mathbf{R^{160}}$;

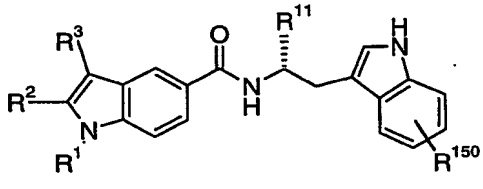
wherein $\mathbf{R^{160}}$ is defined as 1 or 2 substituents selected from: tetrazole, halogen, CN, C_{1-6}alkyl, haloalkyl, $COOR^{161}$, SO_3H, SR^{161}, SO_2R^{161}, OR^{161}, $N(R^{162})_2$, $SO_2N(R^{162})_2$, or $CON(R^{162})_2$, wherein $\mathbf{R^{161}}$ and each $\mathbf{R^{162}}$ is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl; or both $\mathbf{R^{162}}$ are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or a salt thereof;

wherein said probe comprises a detectable label attached to any suitable position, whereby said probe binds to an HCV polymerase or an analog thereof and is capable of being displaced by an inhibitor thereof.

4. The method according to claim 3, wherein said probe is a compound having the following formula:


(IIa),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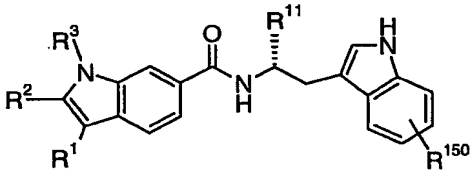
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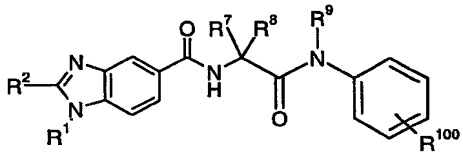
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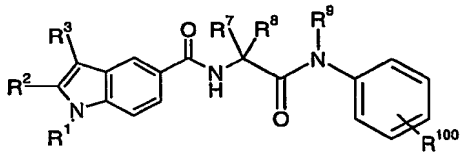
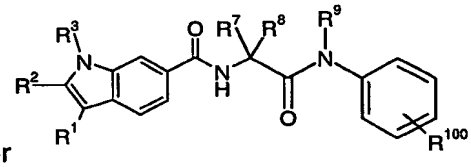

(IIe) or


(IIf).

wherein R^1 is (C_{5-6})cycloalkyl;

R^2 is phenyl, or **Het** both being optionally substituted with R^{20};

R^3, R^7, R^8 ,R^9, R^{100}, and R^{150} are as defined according to claim 2;

R^{11} is OPO_3H, NO_2, cyano, azido, $C(=NH)NH_2$, $C(=NH)NH(C_{1-6})$alkyl or $C(=NH)NHCO(C_{1-6})$alkyl; or

a) (C_{1-6}) alkyl substituted with R^{150a}, haloalkyl, (C_{3-7})cycloalkyl, C_{3-7} spirocycloalkyl optionally containing 1 or 2 heteroatom, (C_{2-6})alkenyl, (C_{2-8})alkynyl, (C_{1-6}) alkyl-(C_{3-7})cycloalkyl, all of which optionally substituted with R^{150}, wherein R^{150a} is the same as R^{150} but is not halogen, OR^{150b}, $COOR^{150b}$, $N(R^{150b})_2$, wherein R^{150b} is H or C_{1-6}alkyl;

b) OR^{104} wherein R^{104} is (C_{1-6}alkyl) substituted with R^{150}, (C_{3-7})cycloalkyl, or

(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with $\mathbf{R^{150}}$;

c) $OCOR^{105}$ wherein R^{105} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

d) SO_3H, $SO_2N(R^{108})_2$ or $SO_2N(R^{108})C(O)R^{108}$ wherein each R^{108} is independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or both R^{108} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** or heterocycle being optionally substituted with R^{150};

e) $NR^{111}R^{112}$ wherein R^{111} is (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, and R^{112} is H, CN, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl, $(C_{1-6}$alkyl)**Het** or

R^{111} is H and R^{112} is SO_2R^{115} wherein R^{115} is (C_{1-6})alkyl, (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or both R^{111} and R^{112} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or heterocycle being optionally substituted with R^{150};

f) $NR^{116}COR^{117}$ wherein R^{116} and R^{117} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, said (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het** being optionally substituted with R^{150};

g) $NR^{118}CONR^{119}R^{120}$, wherein R^{118}, R^{119} and R^{120} is each H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-6}$alkyl)**Het**, or R^{118} is covalently bonded to R^{119} and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

or R^{119} and R^{120} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle;

said alkyl, cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, $(C_{1-6}$alkyl)aryl or $(C_{1-}$

$_6$alkyl)**Het** or heterocycle being optionally substituted with R^{150};

h) $NR^{121}COCOR^{122}$ wherein R^{121} and R^{122} is each is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, a 6- or 10-membered aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150}; or R^{122} is OR^{123} or $N(R^{124})_2$ wherein R^{123} and each R^{124} is independently H, (C_{1-6}alkyl), (C_{3-7})cycloalkyl, or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or R^{124} is OH or O(C_{1-6}alkyl) or both R^{124} are covalently bonded together to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

i) COR^{127} wherein R^{127} is H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl or (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said alkyl, cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

j) $COOR^{128}$ wherein R^{128} is H or (C_{1-6})alkyl substituted with R^{150}, (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, said (C_{3-7})cycloalkyl, or(C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl and (C_{1-6}alkyl)**Het** being optionally substituted with R^{150};

k) $CONR^{129}R^{130}$ wherein R^{129} and R^{130} are independently H, (C_{1-6})alkyl, (C_{3-7})cycloalkyl, (C_{1-6})alkyl-(C_{3-7})cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, or both R^{129} and R^{130} are covalently bonded together and to the nitrogen to which they are attached to form a 5, 6 or 7-membered saturated heterocycle, said alkyl, cycloalkyl, alkyl-cycloalkyl, aryl, **Het**, (C_{1-6}alkyl)aryl, (C_{1-6}alkyl)**Het** and heterocycle being optionally substituted with R^{150};

l) aryl, **Het**, (C_{1-6}alkyl)aryl or (C_{1-6}alkyl)**Het**, all of which being optionally substituted with R^{150}

wherein R^{150} is as defined in claim 3;

or a salt thereof;

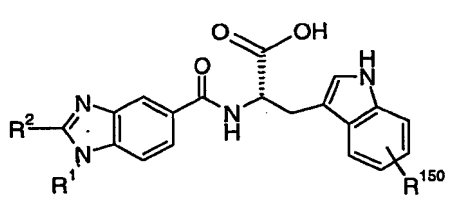
wherein said compound is optionally:

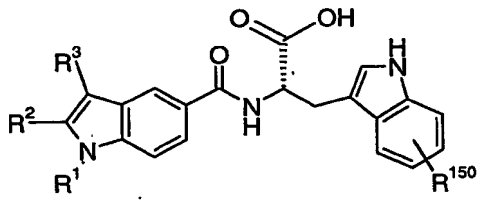
a) marked with a radioactive isotope at any suitable position,

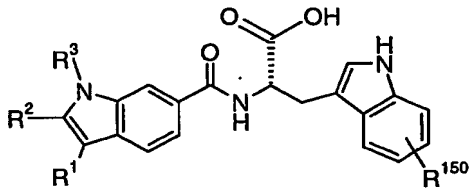
b) linked to a detectable moiety by a suitable linker suitable position, except R^1 and R^3; or

c) linked to an affinity tag at any suitable position, except R^1 and R^3.

5. The method according to claim 4, wherein said probe is a compound having the following formula:


(IIa),


(IIb),


(IIc).

wherein R^1 is (C_{5-6})cycloalkyl;

R^2 is phenyl, or **Het** both being optionally substituted with R^{20};

R^3 and R^{150} are as defined in claim 4;

or a salt thereof;

wherein said compound is optionally:

a) marked with a radioactive isotope at any suitable position;

b) linked to a detectable moiety by a suitable linker at any suitable position, except R^1 and R^3; or

c) linked to an affinity tag at any suitable position, except R^1 and R^3.

6. The method according to claim 2 wherein the detectable label selected from the group consisting of: a fluorescent label a radioactive atom, a chemiluminescent label, and a colorimetric label.

7. The method according to claim 6 wherein the label is a fluorescent label or chemiluminescent label.

8. The method according to claim 7, wherein the fluorescent label is selected from the group consisting of: fluorescein, Oregon green, dansyl, rhodamine, Texas-red, phycoerythrin and Eu^{3+}.

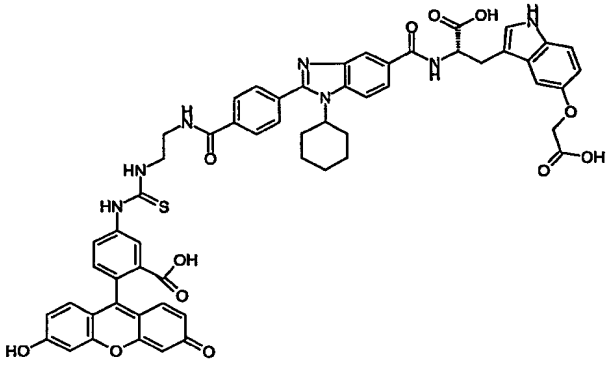
9. The method according to claim 8, wherein the fluorescent label is fluorescein.

10. The method according to claim 2, wherein the detectable label is a fluorescent reporter/quencher pair.

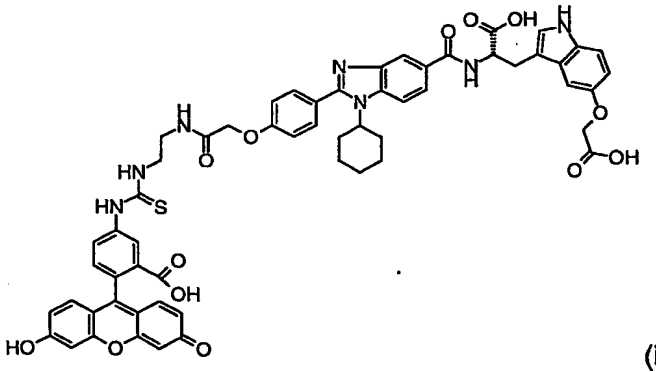
11. The method according to claim 10, wherein the reporter/quencher pair is selected from the group consisting of: EDANS/DABCYL, tryptophan/2,4-dinitrophenyl, tryptophan/DANSYL, 7-methoxycoumarin/2,4-dinitrophenyl, 2-aminobenzoyl/2,4-dinitrophenyl and 2-aminobenzoyl/3-nitrotyrosine.

12. The method according to claim 6, wherein the radioactive atom is selected from ^{3}H, ^{14}C and ^{125}I.

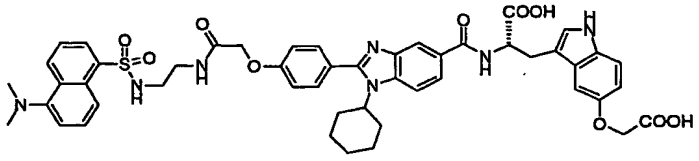
13. The method according to claim 2, wherein the probe is selected from::



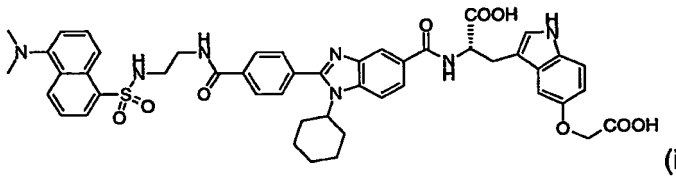
(i);



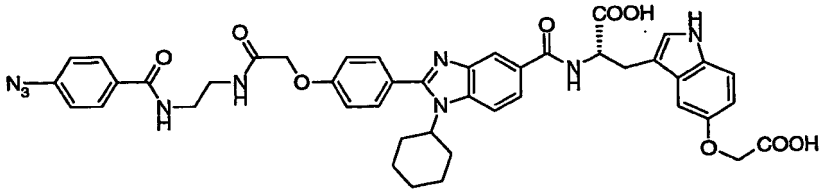
(ii);



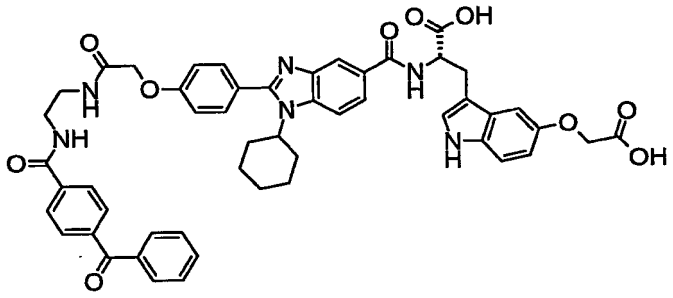
(iii);



(iv);



(v); and



(vi)

14. Use of a probe of formula I, according to claim 2, in the development of an assay for identifying inhibitors of HCV polymerase.

15. A method for identifying compounds that inhibit HCV polymerase comprising the steps of:

a) contacting said HCV polymerase or an analog thereof with a probe of formula I, according to claim 2, so as to form a complex having said probe bound to said polymerase;

b) measuring the signal from said complex to establish a base line level;

c) incubating the product of step a) with a test compound; and

d) measuring the signal from said complex; and

e) comparing the signal from step d) with the signal from step b);

whereby a modulation in said signal is an indication that said test compound inhibits said polymerase.

16. A method for identifying compounds capable of inhibiting HCV polymerase,

comprising:

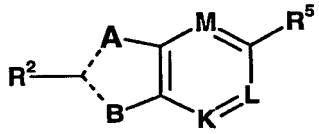
f) repeating steps (a) to (e), according to claim 15, in a high throughput screen.

17. The method according to claim 15, wherein the HCV polymerase is selected from the group consisting of: NS5B; NS5BΔ21; and NS5BΔ57 or analogs thereof.

18. The method according to claim 15, wherein the HCV polymerase is obtained from genotype HCV-1a or HCV-1b strains optionally having a histidine tag at either the N- or C-terminal.

19. A kit for testing compounds potentially binding to HCV polymerase, said kit comprising the probe of formula (I) according to claim 2, and instructions on how to use said probe for identifying test compounds binding to said polymerase.

20. A probe of formula I:



I

A is O, S, NR^3, or CR^3;

B is NR^1 or CR^1; with the proviso that, when **A** is CR^3, **B** is NR^1, and when **A** is O or S, **B** is CR^1;

----- represents either a single or a double bond;

R^1 is selected from the group consisting of: (C_{4-7})cycloalkyl optionally substituted with $(C_{1-6}$ alkyl); norbornane, 5-, 6- or 7-membered heterocycle having 1 to 4 heteroatoms selected from O, N, and S, all of which optionally substituted with 1 to 4 substituent selected from the group consisting of:

halo, OH and C_{1-6} alkyl optionally substituted with hydroxy;

R^2 is selected from the group consisting of: phenyl, pyridine-N-oxide, 5- or 6-membered aromatic heterocycle having 1 to 4 heteroatoms selected from O, N, and S, and 9- or 10-membered aromatic heterobicycle having 1 to 4 heteroatoms selected from O, N and S;

said phenyl, pyridine-N-oxide, aromatic heterocycle and aromatic heterobicycle being optionally substituted with from 1 to 4 substituents selected from the group consisting of: halogen, C_{1-6} haloalkyl, (C_{1-6})alkyl, C_{1-6} alkoxy, OH, amino optionally mono- or di-substituted with C_{1-6} alkyl;

R^3 is selected from the group consisting of: H, (C_{1-6})alkyl, (C_{1-6} alkyl)-(C_{6-10}aryl), (C_{1-6} alkyl)-5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N, and S, and 5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N and S,

wherein said aryl and said heterocycle are optionally substituted with from 1 to 4 substituents selected from the group consisting of: COOH, COO(C_{1-6} alkyl), halogen, and (C_{1-6} alkyl);

M is N, CR^{4a}, or COR^{4b}, wherein R^{4a} is selected from the group consisting of: H, halogen, and (C_{1-6} alkyl); and R^{4b} is selected from the group consisting of: H and (C_{1-6} alkyl);

K and **L** is each independently N or CR^6, wherein R^6 is H, halo, C_{1-6} alkyl, OH, or C_{1-6} alkoxy;

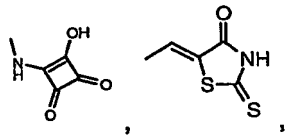
R^5 is -C(Y)-Z, wherein **Y** is O or S; and **Z** is NHR^{5a} or OR^{5a};

wherein:

R^{5a} is selected from the group consisting of: H, (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{3-6})cycloalkyl optionally substituted with C_{1-6}alkyl or C_{2-6}alkenyl, (C_{6-10})aryl optionally substituted with C_{1-6}alkyl or C_{2-6}alkenyl, N{(C_{1-6}) alkyl}$_2$, NHCOO(C_{1-6})alkyl(C_{6-10})aryl, NHCO(C_{6-10})aryl, -5- or 6-atom heterocycle, having 1 to 4 heteroatoms selected from O, N and S, and -9- or 10-atom heterobicycle having 1 to 4 heteroatoms selected from O, N and S;

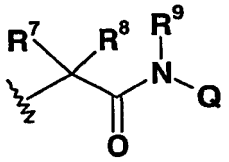
wherein said alkyl, alkenyl, cycloalkyl, aryl, heterocycle or heterobicycle are all optionally substituted with from 1 to 4 substituents selected from: OH, COOH, (C_{1-6})alkyl, (C_{2-4})alkenyl, (C_{1-6})alkyl-hydroxy, COO(C_{1-6})alkyl, C_{3-7} cycloalkyl, benzyloxy, halogen, (C_{2-4})alkenyl-(C_{1-6})alkyl-COOH, coumarin, (C_{1-6})alkyl-amino, NH(C_{1-6} alkyl), C(halogen)$_3$, -C(O)NH(C_{1-4})alkyl, and -C(O)NH(C_{6-10})aryl, 5- or 6-membered heterocycle having 1 to 4 heteroatoms selected from O, N and S, 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N and S, and 6- or 10-membered aryl;

wherein said alkyl, alkenyl, cycloalkyl, aryl, heterocycle and heterobicycle are all optionally substituted with from 1 to 4 substituents selected from: halogen, OPO_3H, sulfonamido, SO_3H, SO_2CH_3, $-CONH_2$, $-COCH_3$, (C_{1-3})alkyl, $(C_{2-4}$alkenyl)COOH, tetrazolyl, COOH, $-CONH_2$, triazolyl, OH, NO_2, NH_2, $-O(C_{1-6}$ alkyl)COOH, hydantoin, benzoyleneurea, (C_{1-4})alkoxy, cyano, azido, $-O-(C_{1-6})$alkyl COOH, $-O-(C_{1-6})$alkyl COO-(C_{1-6})alkyl, NHCO-$(C_{1-6}$alkyl), -NHCOCOOH, -NHCOCONHOH,-$NHCOCONH_2$, $-NHCOCONHCH_3$, $-NHCO(C_{1-6})$alkyl-COOH, $-NHCOCONH(C_{1-6})$alkyl-COOH, $-NHCO(C_{3-7})$cycloalkyl-COOH, $-NHCONH(C_{6-10})$aryl-COOH, $-NHCONH(C_{6-10})$aryl-COO(C_{1-6})alkyl, - $NHCONH(C_{1-6})$alkyl-COOH,- $NHCONH(C_{1-6})$ alkyl-COO(C_{1-6})alkyl, - $NHCONH(C_{1-6})$alkyl-(C_{2-6})alkenyl-COOH, - $NH(C_{1-6})$alkyl-(C_{6-10})aryl-O(C_{1-6})alkyl COOH, - $NH(C_{1-6})$alkyl-(C_{6-10})aryl-COOH, $-NHCH_2COOH$, $-NHCONH_2$, $-NHCO(C_{1-6})$hydroxyalkyl COOH, $-OCO(C_{1-6})$hydroxyalkyl COOH, (C_{3-6})cycloalkyl COOH,



, -NHCN, -NHCHO, $-NHSO_2CH_3$, $-NHSO_2CF_3$; and $-O(C_{1-6}$alkyl)-tetrazol;

or R^{5a} is



wherein R^7 and R^8 are each independently H, $(C_{1-6}$ alkyl), $(C_{3-7}$ cycloalkyl), $(C_{1-6}$ alkyl)phenyl, $(C_{1-6}$ alkyl)-$(C_{3-7}$ cycloalkyl), $(C_{3-7}$ cycloalkyl)-(C_{1-6} alkyl), $(C_{3-7}$ cycloalkyl)-$(C_{2-4}$ alkenyl), $(C_{1-6}$ alkyl)-OH, phenyl, CH_2biphenyl, 5- or 6-membered heterocycle having from1 to 4 heteroatoms selected from O, N, and S, 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N, and S, $(C_{1-6}$ alkyl)-5- or 6-membered heterocycle having from1 to 4 heteroatoms selected from O, N, and S, or $(C_{1-6}$ alkyl)-9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N, and S,

or R^7 and R^8 are covalently bonded together to form $(C_{3-7}$ cycloalkyl), 4-, 5- or

6-membered heterocycle having from1 to 4 heteroatoms selected from O, N, and S; or one of R^7 or R^8 is covalently bonded to R^9 to form a pyrrolidine;

wherein said alkyl, cycloalkyl, heterocycle, heterobicycle, phenyl are optionally substituted with from 1 to 4 substituents selected from the group consisting of: OH, COOH, (C_{1-6} alkyl), (C_{2-4} alkenyl), $CONH_2$, NH_2, $NH(C_{1-6}$ alkyl), $N(C_{1-6}$ alkyl)$_2$, NHCOCOOH, $NHCOCON(C_{1-6}$ alkyl)$_2$, $NHCOCONH(C_{1-6}$ alkyl), SH, $S(C_{1-6}$ alkyl), $NHC(=NH)NH_2$, halogen, and $COO(C_{1-6}$alkyl);

R^9 is H or (C_{1-6} alkyl); and

Q is selected from the group consisting of: (C_{1-3}alkyl)CONHaryl, 6- or 10-membered aryl, biphenyl, 5- or 6-atom heterocycle having 1 to 4 heteroatoms selected from O, N and S, 9- or 10-membered heterobicycle having 1 to 4 heteroatoms selected from O, N and S;

wherein said aryl, biphenyl, heterocycle and heterobicycle are all optionally substituted with from 1 to 4 substituents selected from: OH, COOH, $COO(C_{1-6})$alkyl, (C_{1-6})alkyl, (C_{1-6})alkylCOOH, (C_{1-6} alkyl)(C_{2-4} alkynyl), (C_{1-6})alkyl-hydroxy, phenyl, benzyloxy, halogen, (C_{2-4})alkenyl, (C_{2-4})alkenyl-(C_{1-6})alkyl-COOH, 5- or 6-membered second heterocycle having 1 to 4 heteroatoms selected from O, N and S, NH-5- or 6- membered second heterocycle having 1 to 4 heteroatoms selected from O, N, and S,

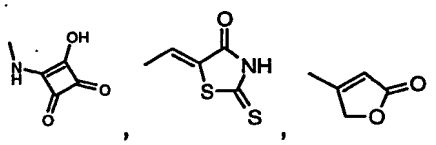
wherein said second heterocycle and phenyl being optionally substituted with from 1 to 4 substituents selected from: (C_{1-6} alkyl), CF_3, OH, (C_{1-6}alkyl) COOH, $O(C_{1-6}$alkyl)COOH, (C_{1-6}alkyl) $COO(C_{1-6}$alkyl), CH_2phenyl, $COO(C_{1-6}$ alkyl), (C_{1-6}alkyl)$O(C_{1-6}$alkyl), COOH, $NCH(C_{1-6}$alkyl)$_2$, $NCO(C_{1-6}$ alkyl), NH_2, $NH(C_{1-6}$ alkyl), halogen, $N(C_{1-6}$ alkyl)$_2$; and C_{2-6} alkenyl-COOH

halogen, OPO_3H, benzyl, sulfonamido, SH, $SOCH_3$, SO_3H, SO_2CH_3, $S(C_{1-6}$ alkyl)COOH, $-CONH_2$, $-COCH_3$, (C_{1-3})alkyl, (C_{2-4}alkenyl)COOH

wherein said alkenyl is optionally substituted with from 1 to 2 (C_{1-6} alkyl) substituents,

(C_{2-4}alkenyl)$COO(C_{1-6}$alkyl), tetrazolyl, COOH, triazolyl, OH, NO_2, NH_2, , -$O(C_{1-6}$ alkyl)COOH, hydantoin, benzoyleneurea, (C_{1-4})alkoxy, (C_{1-4})alkoxy(C_{1-6} alkyl)COOH, cyano, azido, -O-(C_{1-6})alkyl COOH, -O-(C_{1-6})alkyl COO-(C_{1-6})alkyl, -NHCOCOOH, -NHCOCONHOH,-$NHCOCONH_2$, $-NHCOCONHCH_3$, $-NHCO(C_{1-6})$alkyl-COOH, $-NHCOCONH(C_{1-6})$alkyl-COOH, $-NHCO(C_{3-7})$cycloalkyl-COOH, $-NHCONH(C_{6-10})$aryl-COOH, - $NHCONH(C_{6-}$

$_{10}$)aryl-COO(C_{1-6})alkyl, - NHCONH(C_{1-6})alkyl-COOH,- NHCONH(C_{1-6})alkyl-COO(C_{1-6})alkyl, - NHCONH(C_{1-6})alkyl-(C_{2-6})alkenyl-COOH, - NH(C_{1-6})alkyl-(C_{6-10})aryl-O(C_{1-6})alkyl COOH, - NH(C_{1-6})alkyl-(C_{6-10})aryl-COOH, -NHCH$_2$COOH, -NHCONH$_2$, -NHCO(C_{1-6})hydroxyalkyl COOH, -OCO(C_{1-6})hydroxyalkyl COOH, (C_{3-6})cycloalkyl COOH,



, -NHCN, -NHCHO, -NHSO$_2$CH$_3$, -NHSO$_2$CF$_3$, coumarin, (C_{1-6})alkyl-amino, NH(C_{1-6}alkyl)$_2$, C(halogen)$_3$, -NH(C_{2-4})acyl, -NH(C_{6-10})aroyl, -CONH(C_{1-6}alkyl), -CO(C_{1-6})alkyl-COOH, -CONH(C_{1-6})alkyl-COOH, -CO-NH-alanyl, -CONH(C_{2-4})alkylN(C_{1-6}alkyl)$_2$, -CONH(C_{2-4}) alkyl-Het, -CONH(C_{2-4}) alkyl-(COOH)-Het, -CONH(C_{1-2} alkyl)(OH)(C_{1-2} alkyl)OH, -CONH(C_{1-6}) alkyl-COOH, -CONH(C_{6-10} aryl), -CONH-Het, -CONH(C_{6-10}) aryl-COOH, -CONH(C_{6-10}) aryl-COO(C_{1-6}) alkyl, -CONH(C_{1-6}) alkyl-COO(C_{1-6}) alkyl, -CONH(C_{6-10}) aryl-(C_{1-6})alkyl-COOH, and -CONH(C_{6-10}) aryl-(C_{2-6})alkenyl-COOH;

or a salt thereof;

said probe comprises a detectable label, whereby said probe binds to an HCV polymerase or an analog thereof and is capable of being displaced by an inhibitor thereof.

21. A method for identifying compounds that inhibit HCV polymerase comprising the steps of:

a) contacting said HCV polymerase or an analog thereof with a probe of formula I according to claim 20, so as to form a complex having said probe bound to said polymerase;

b) measuring the signal from said complex to establish a base line level;

c) incubating the product of step a) with a test compound; and

d) measuring the signal from said complex; and

e) comparing the signal from step d) with the signal from step b);

whereby a modulation in said signal is an indication that said test compound inhibits said polymerase.

FIGURE 1

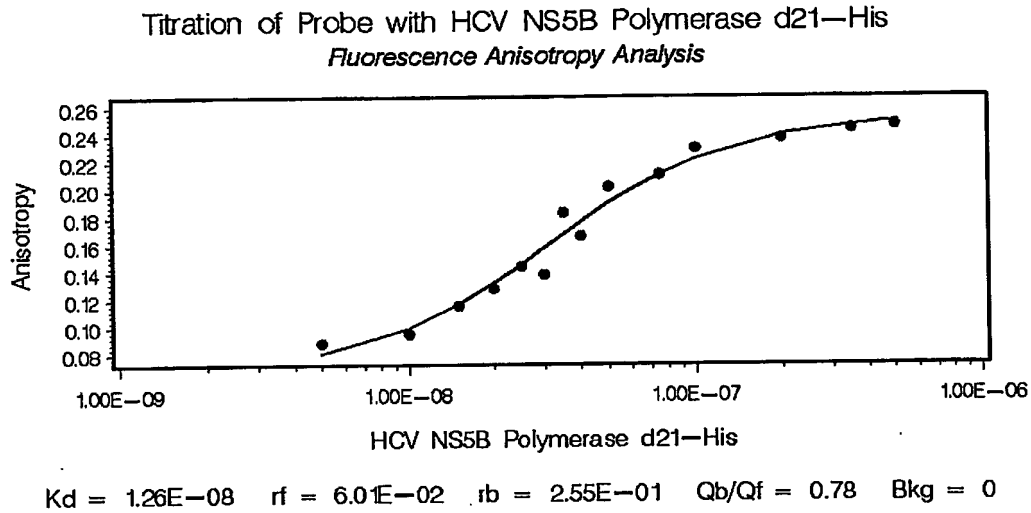


FIGURE 2

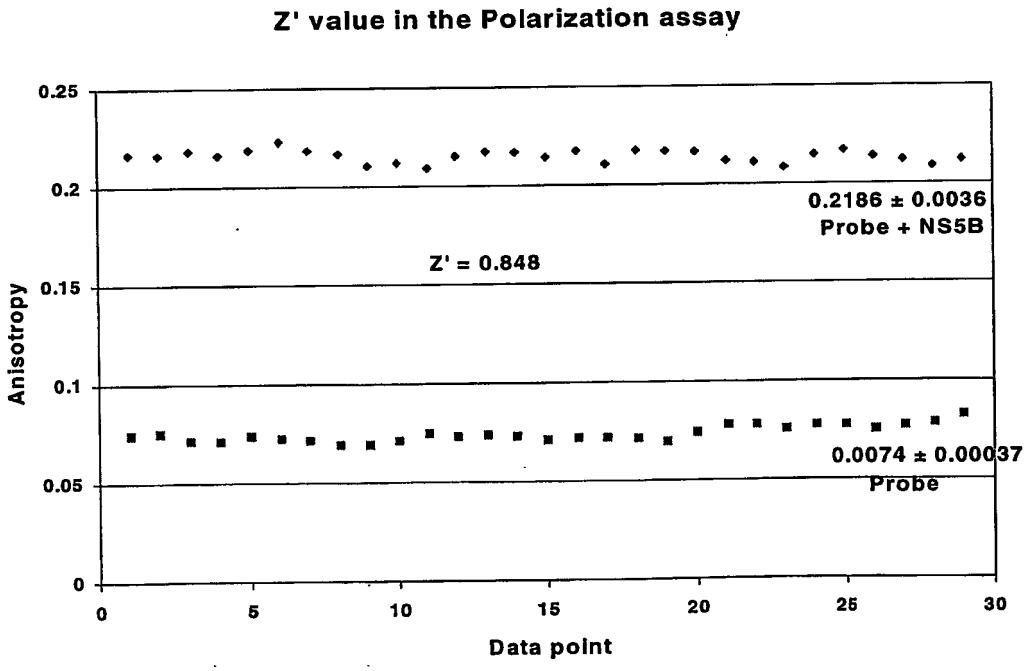
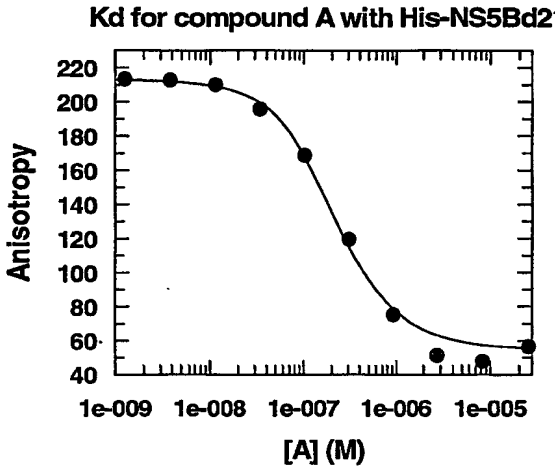


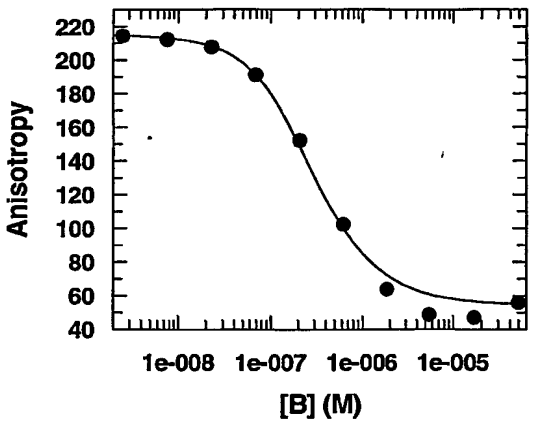
FIGURE 3

A.



Variable	Value	Std. Err.
Kd of inhibitor	3.0971e-008	5.5429e-009
Qb/Qf	6.6898e-001	6.1946e-002

B.

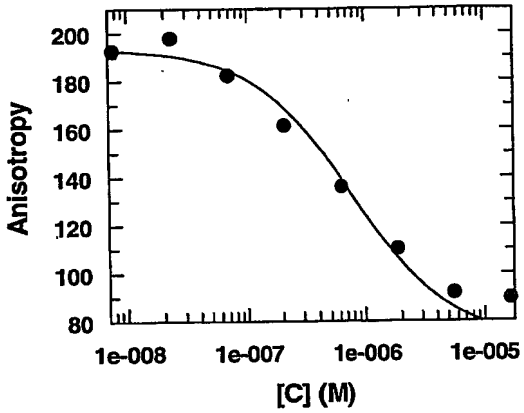


Variable	Value	Std. Err.
Kd of inhibitor	4.1498e-008	8.1777e-009
Qb/Qf	7.1835e-001	8.0672e-002

FIGURE 4

A.

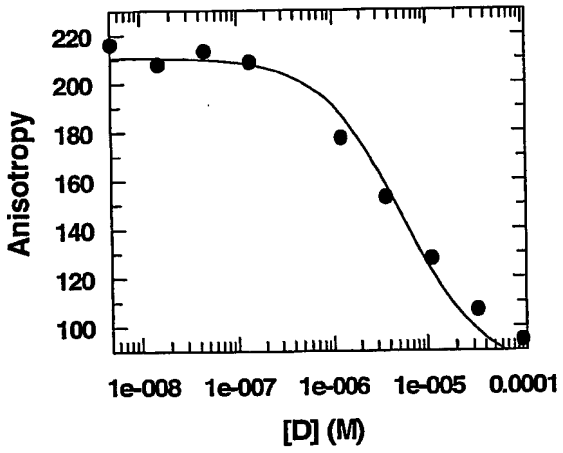
Kd for compound C with NS5Bd21-His



Variable	Value	Std. Err.
Kd of inhibitor	2.3067e-007	5.8224e-008
Qb/Qf	7.4124e-001	9.1773e-002

B.

Kd for compound D with NS5Bd21-His

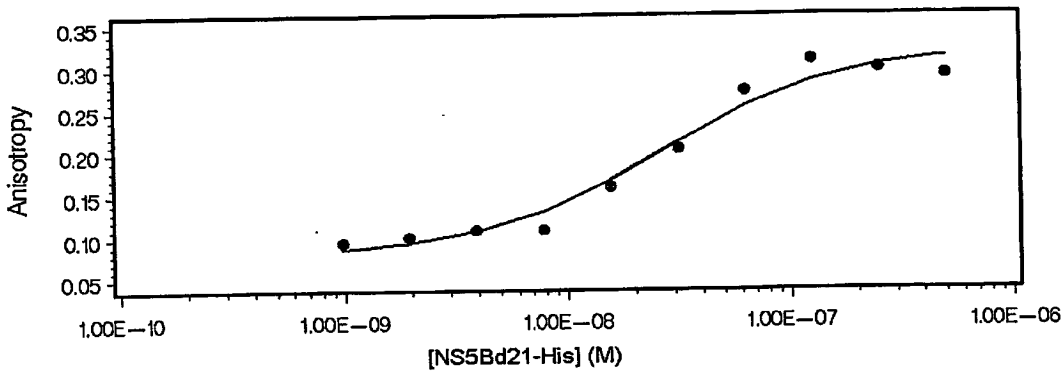


Variable	Value	Std. Err.
Kd of Inhibitor	1.0824e-006	2.3258e-007
Qb/Qf	6.5635e-001	8.2954e-002

FIGURE 5

Titration of probe i with NS5Bd21-His polymerase in Tris pH 7.5 and 30 mM NaCl

Fluorescence Anisotropy Analysis

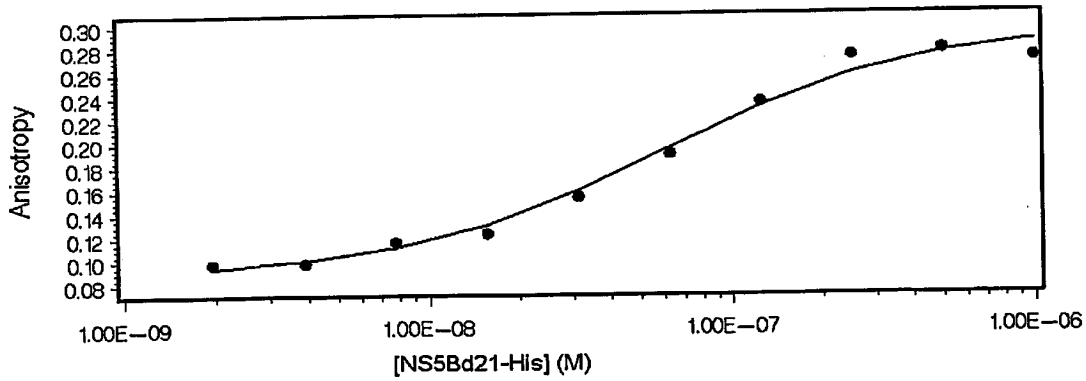


Kd = 1.53E−08 rf = 7.86E−02 rb = 3.23E−01 Qb/Qf = 0.68 Bkg = 0

FIGURE 6

Titration of probe i with NS5Bd21-His polymerase in Tris pH 7.5 and 100 mM NaCl

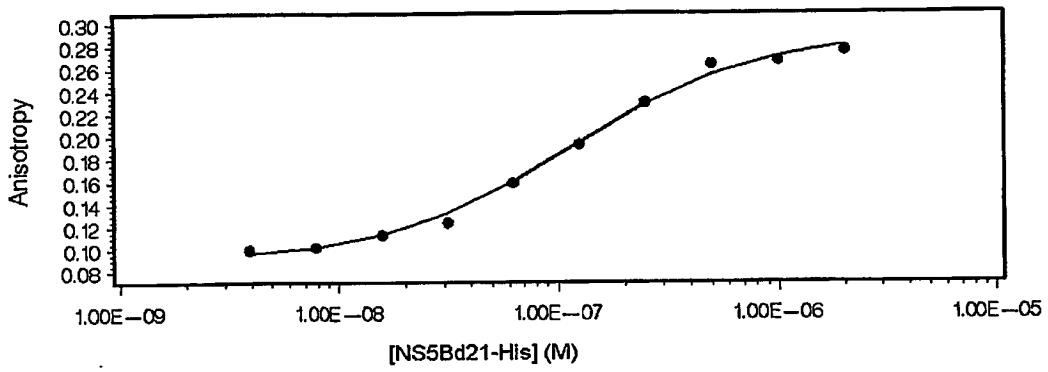
Fluorescence Anisotropy Analysis



Kd = 3.89E−08 rf = 8.79E−02 rb = 2.96E−01 Qb/Qf = 0.7 Bkg = 0

FIGURE 7

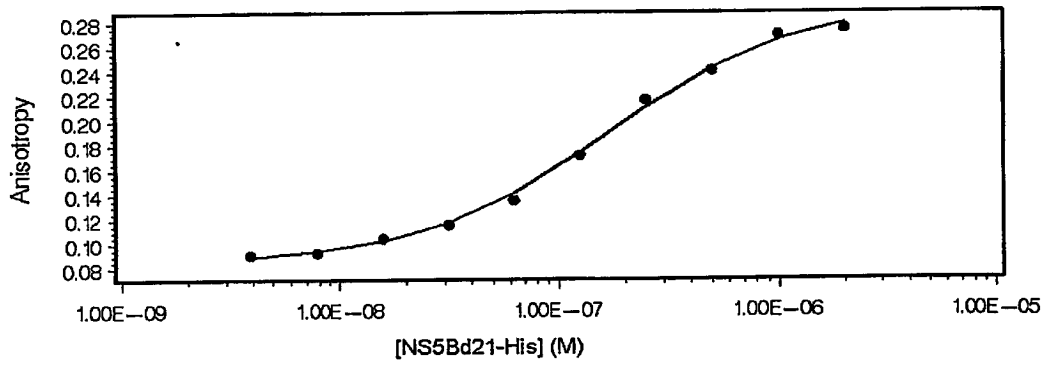
Titration of probe i with NS5Bd21-His polymerase in Tris pH 7.5 and 150 mM NaCl
Fluorescence Anisotropy Analysis



Kd = 7.83E−08 rf = 8.91E−02 rb = 2.91E−01 Qb/Qf = 0.7 Bkg = 0

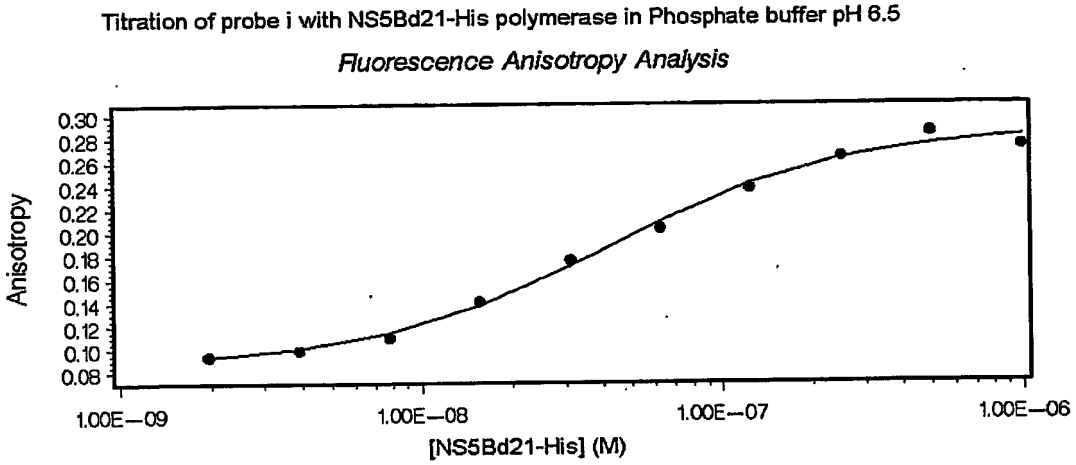
FIGURE 8

Titration of probe i with NS5Bd21-His polymerase in Tris pH 7.5 and 200 mM NaCl
Fluorescence Anisotropy Analysis



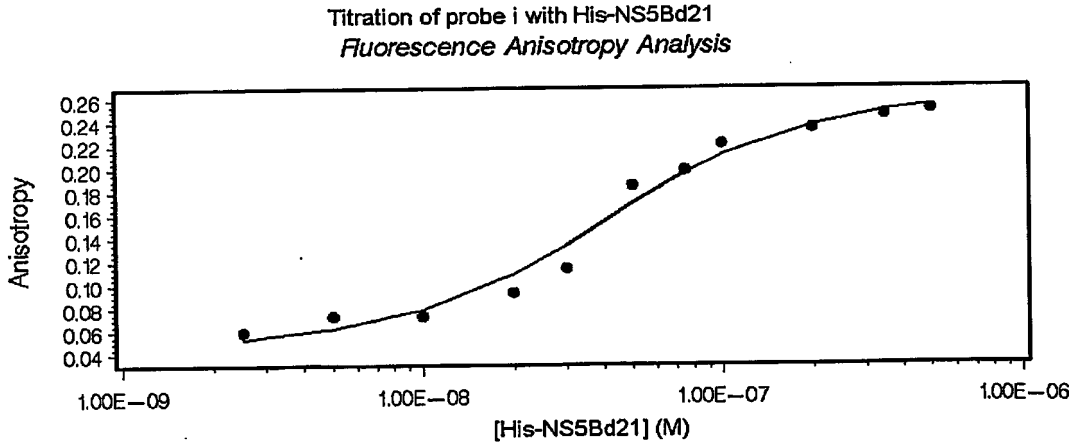
Kd = 1.22E−07 rf = 8.51E−02 rb = 2.96E−01 Qb/Qf = 0.73 Bkg = 0

FIGURE 9



Kd = 3.33E−08 rf = 8.55E−02 rb = 2.88E−01 Qb/Qf = 0.974 Bkg = 0

FIGURE 10

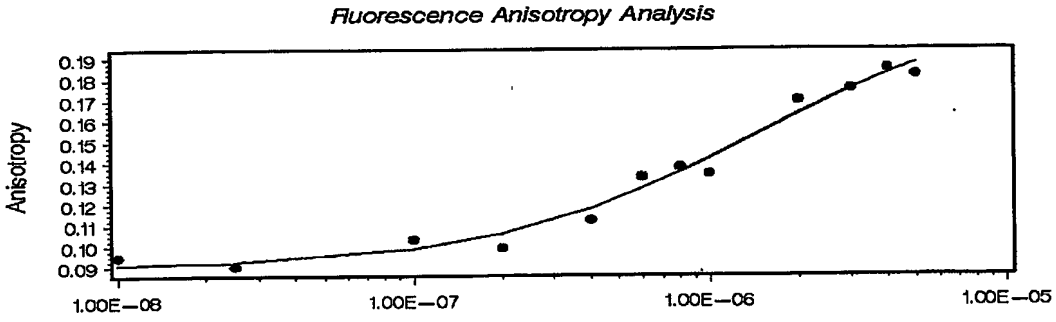


Kd = 1.81E−08 rf = 4.44E−02 rb = 2.64E−01 Qb/Qf = 0.7 Bkg = 0

FIGURE 11

Titration of probe ii with GBV-B polymerase

[GBV-BΔ23-His] (M)

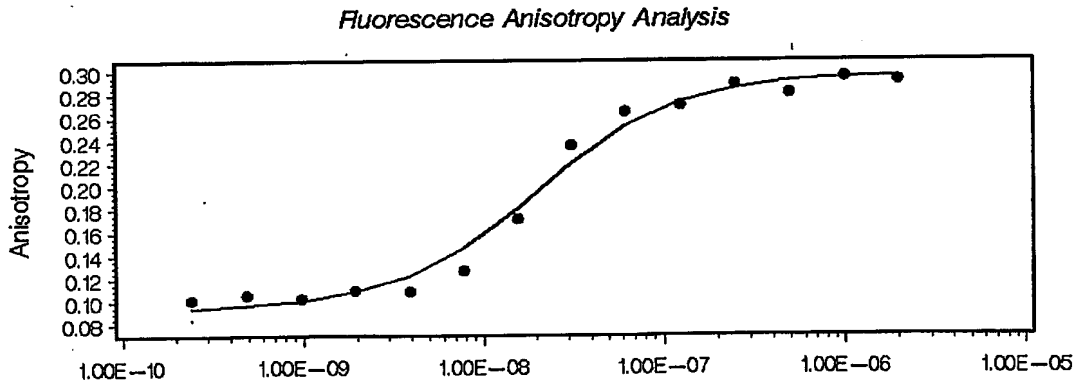


Kd = 1.79E−06 rf = 9.03E−02 rb = 2.14E−01 Qb/Qf = 1.29 Bkg = 0

FIGURE 12

Titration of probe ii with His-NS5BΔ21 (H77c,1a) polymerase

[His-NS5BΔ21(H77c,1a)] (M)



Kd = 1.82E−08 rf = 9.22E−02 rb = 2.97E−01 Qb/Qf = 1.18 Bkg = 0

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